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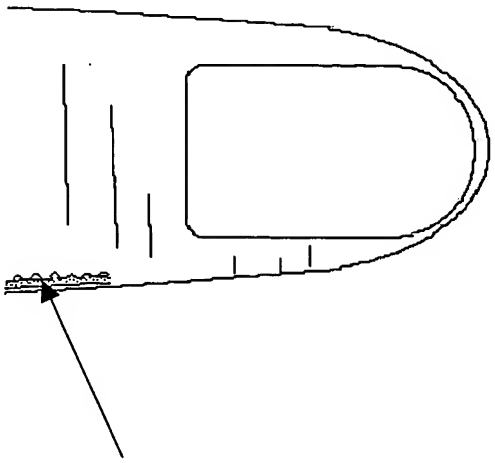

Fingertip
Reporter and Marker colors
Stratum corneum
Stratum granulosum
Stratum germinativum
Dermis
Capillary fields extending into the dermal papillae from subcutaneous arteries and veins

FIG. 1

FIG. 2A

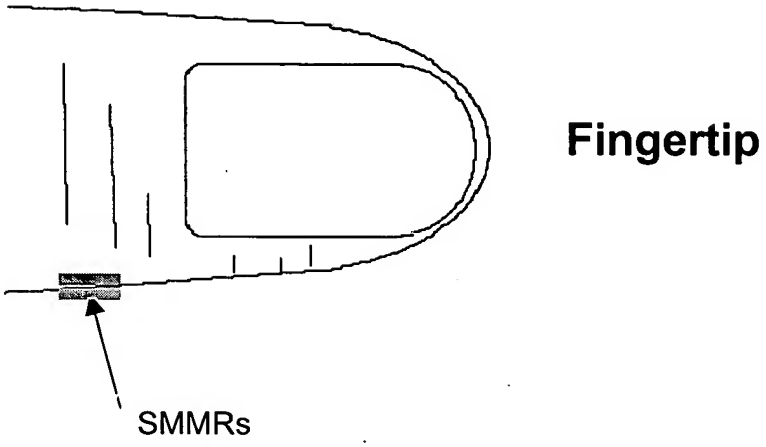

Fingertip
SMMRs

FIG. 2B

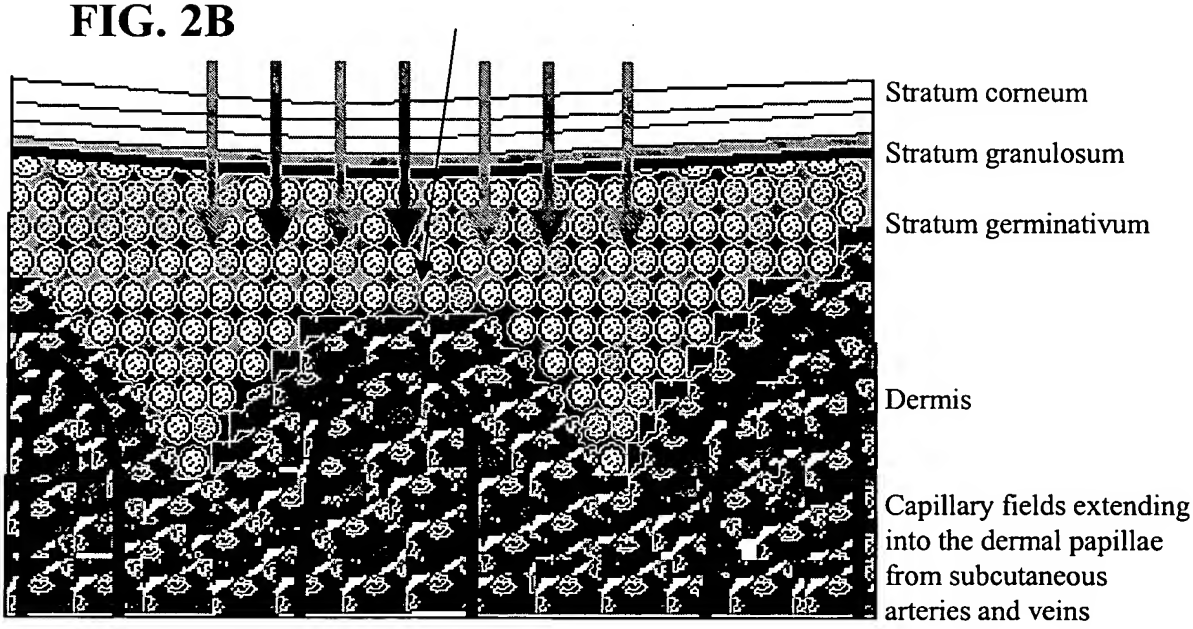

Stratum corneum
Stratum granulosum
Stratum germinativum
Dermis
Capillary fields extending into the dermal papillae from subcutaneous arteries and veins

FIG. 3A

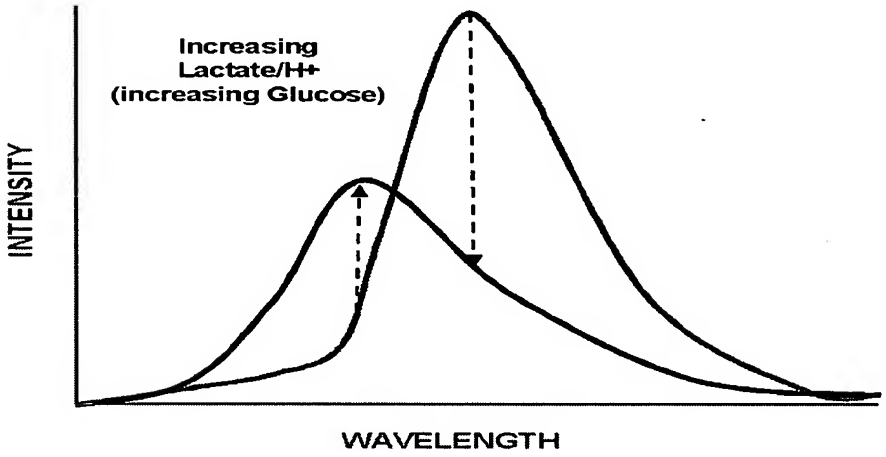

Increasing
Lactate/H+
(increasing Glucose)
INTENSITY
WAVELENGTH

FIG. 3B

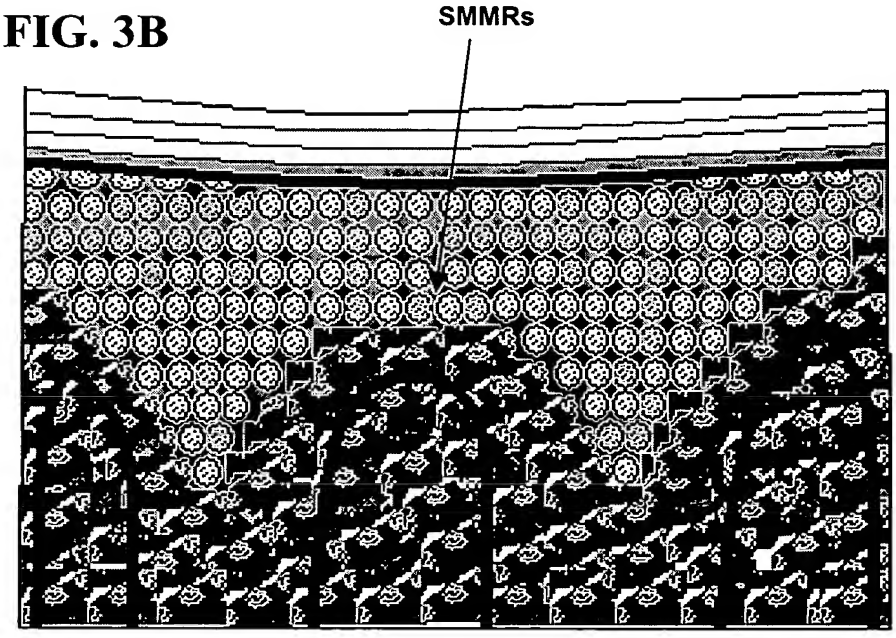
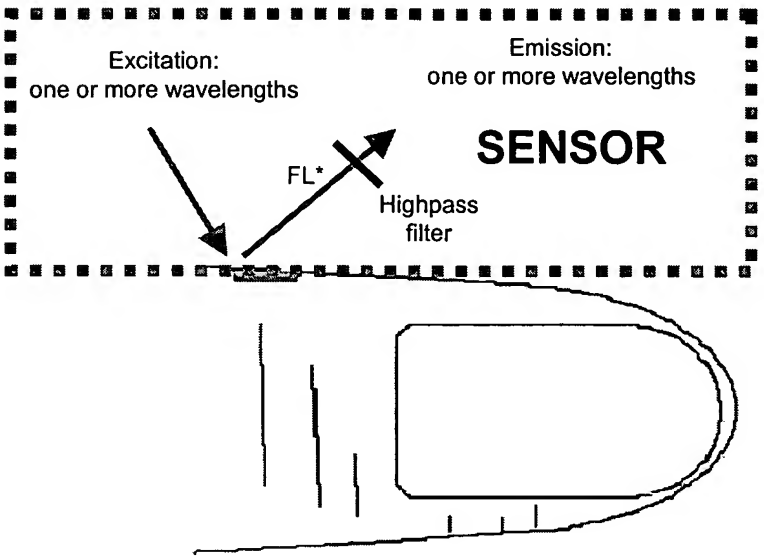

SMMRs
Stratum corneum
Stratum granulosum
Stratum germinativum
Dermis
Capillary fields extending into the dermal papillae from subcutaneous arteries and veins

FIG. 4A



* FL is measured fluorescence

FIG. 4B

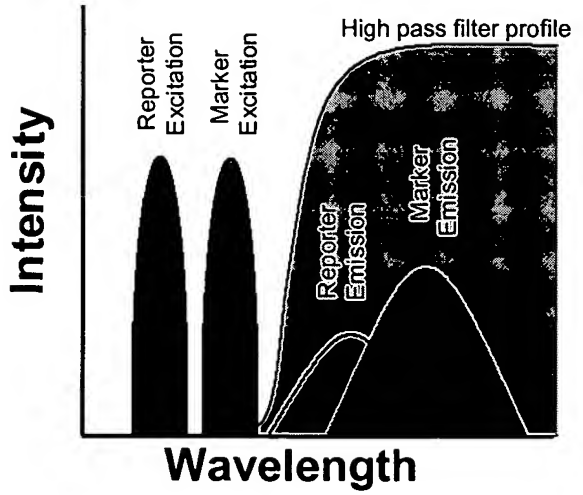
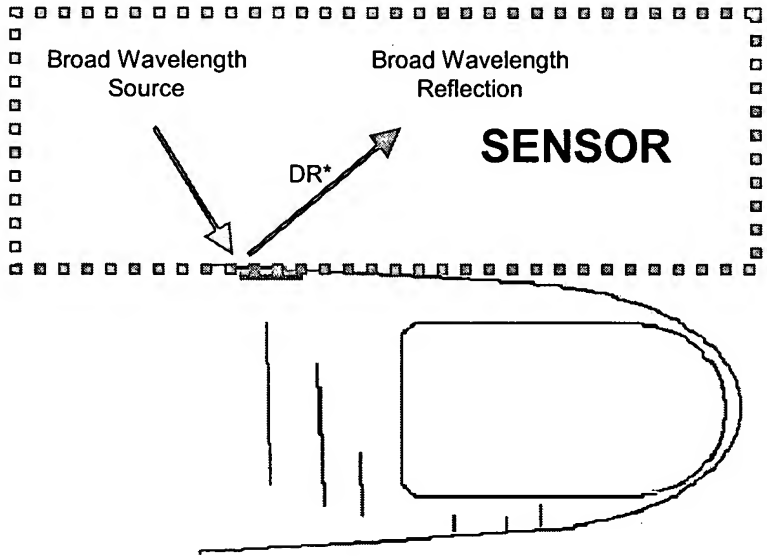
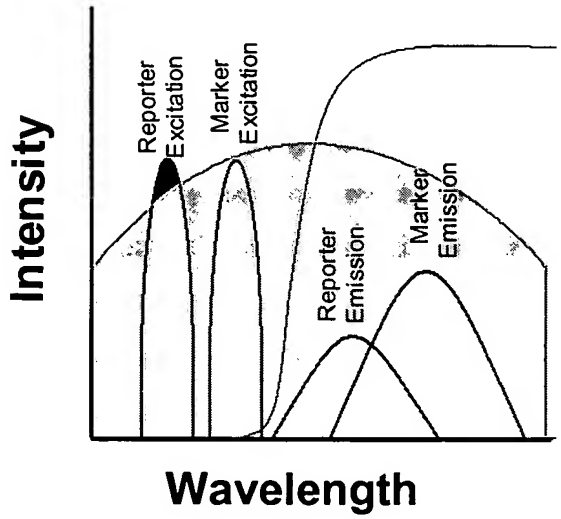


FIG. 5A



* DR is measured diffuse reflection

FIG. 5B



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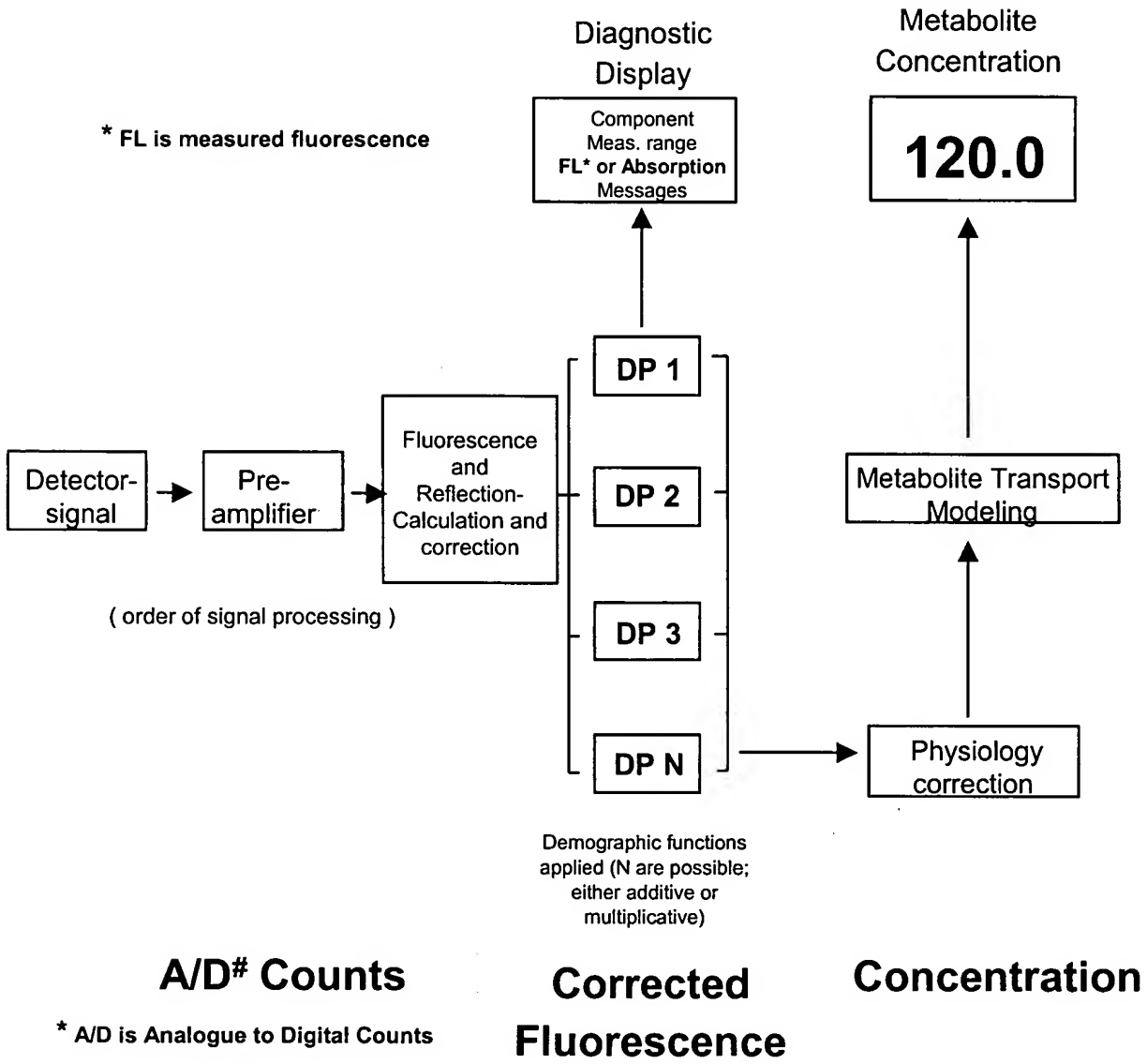
Diagnostic Display
Metabolite Concentration
* FL is measured fluorescence
Component Meas. range FL* or Absorption Messages
120.0
DP 1
DP 2
DP 3
DP N
Detector-signal
Pre-amplifier
Fluorescence and Reflection-Calculation and correction
(order of signal processing)
Metabolite Transport Modeling
Physiology correction
Demographic functions applied (N are possible; either additive or multiplicative)
A/D# Counts
* A/D is Analogue to Digital Counts
Corrected Fluorescence
Concentration

FIG. 6

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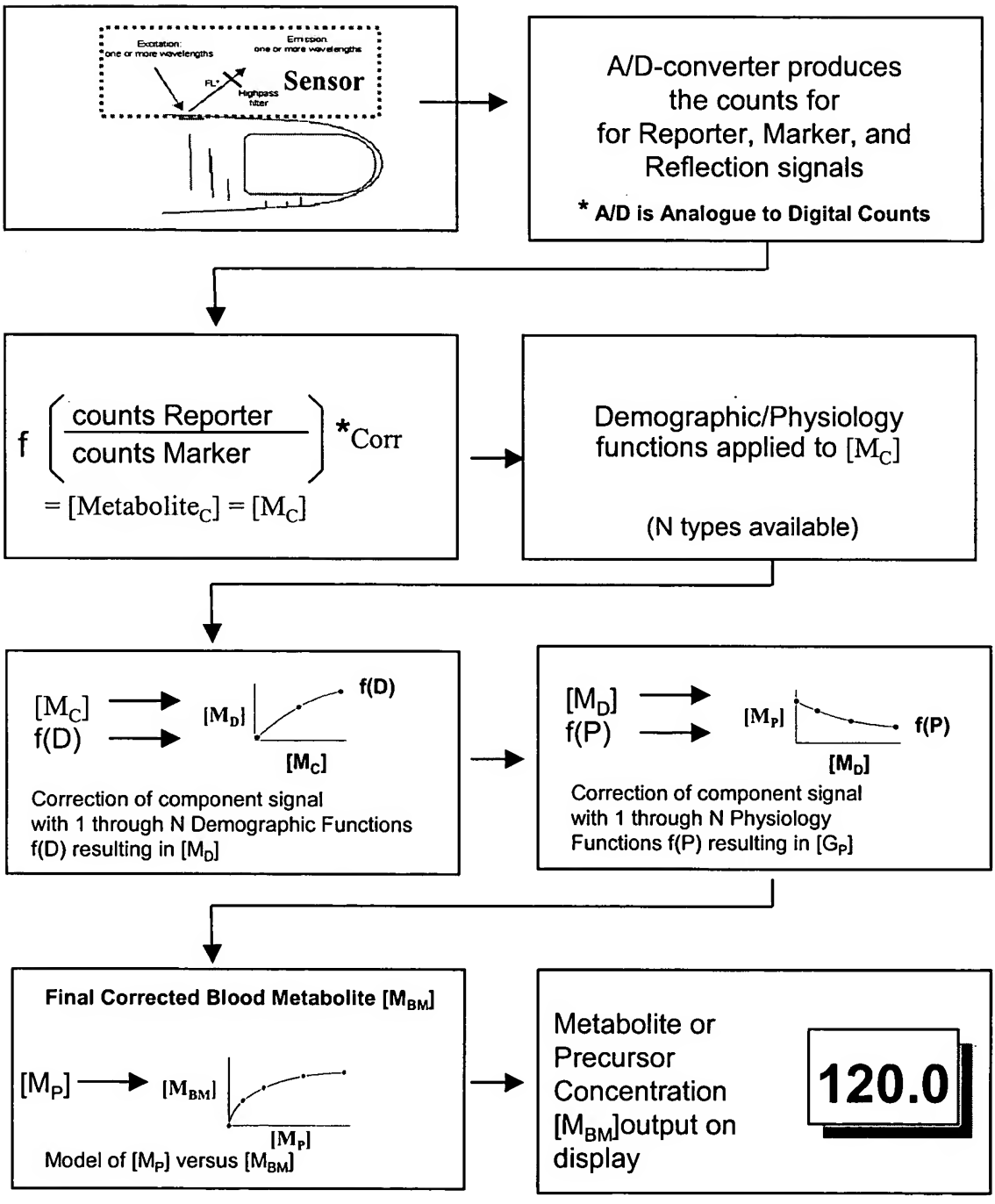

Excitation: one or more wavelengths
Emission one or more wavelengths
FL*
Highpass filter
Sensor
A/D-converter produces the counts for for Reporter, Marker, and Reflection signals
* A/D is Analogue to Digital Counts
f (counts Reporter / counts Marker) *Corr
= [Metabolite$_C$] = [M_C]
Demographic/Physiology functions applied to [M_C]
(N types available)
[M_C]
f(D)
[M_D]
f(D)
[M_C]
Correction of component signal with 1 through N Demographic Functions f(D) resulting in [M_D]
[M_D]
f(P)
[M_P]
f(P)
[M_D]
Correction of component signal with 1 through N Physiology Functions f(P) resulting in [G_P]
Final Corrected Blood Metabolite [M_{BM}]
[M_P]
[M_{BM}]
[M_P]
Model of [M_P] versus [M_{BM}]
Metabolite or Precursor Concentration [M_{BM}]output on display
120.0

FIG. 7

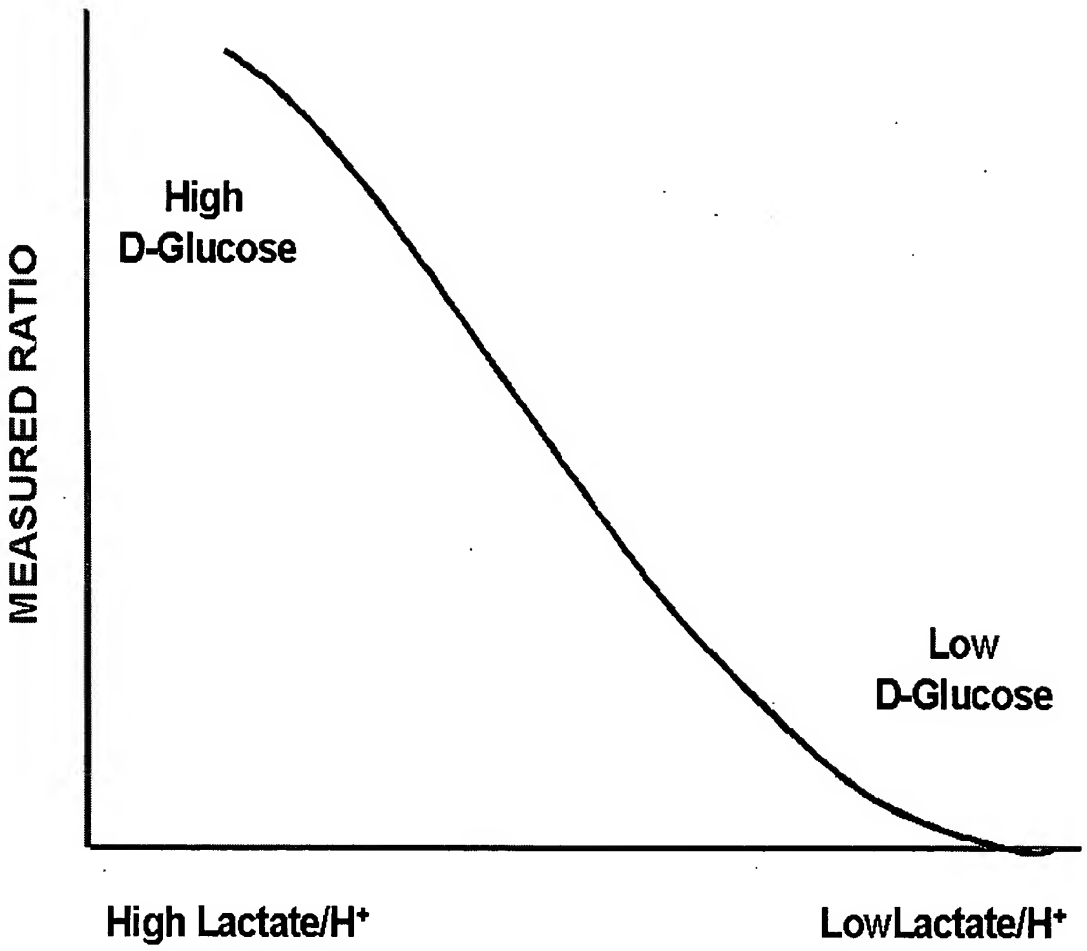

MEASURED RATIO
High
D-Glucose
Low
D-Glucose
High Lactate/H+
LowLactate/H+

FIG. 8

FIG. 9A

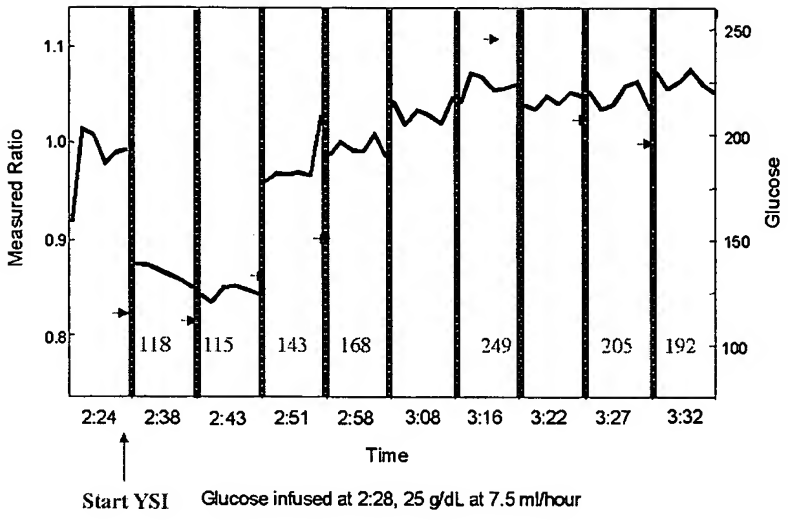

Measured Ratio
1.1
1.0
0.9
0.8
Glucose
250
200
150
100
118
115
143
168
249
205
192
2:24
2:38
2:43
2:51
2:58
3:08
3:16
3:22
3:27
3:32
Time
Start YSI
Glucose infused at 2:28, 25 g/dL at 7.5 ml/hour

FIG. 9B

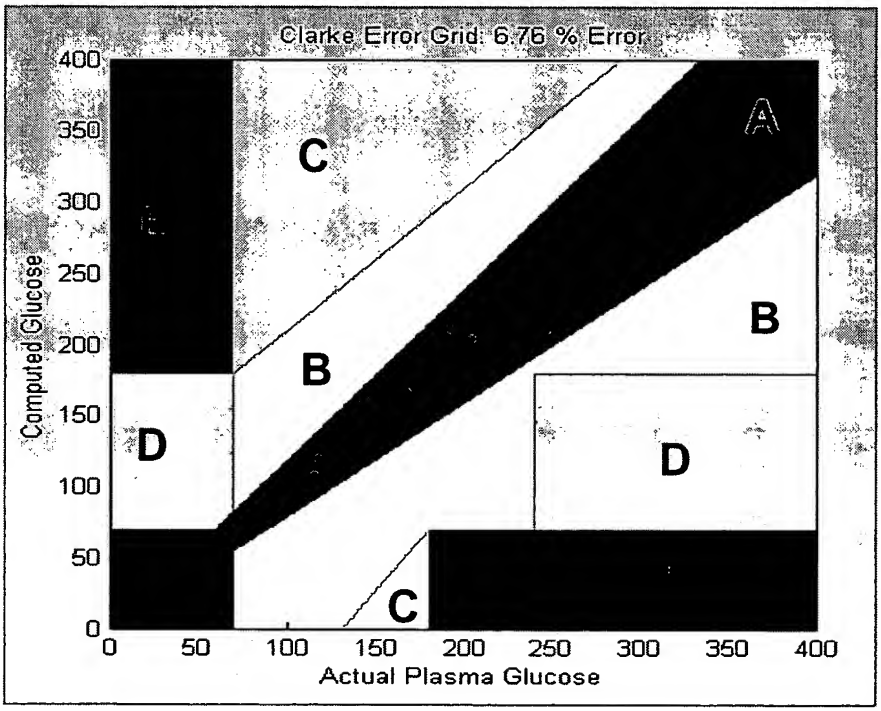

Clarke Error Grid: 6.76 % Error
Computed Glucose
Actual Plasma Glucose
0
50
100
150
200
250
300
350
400
A
B
C
D

FIG. 10A

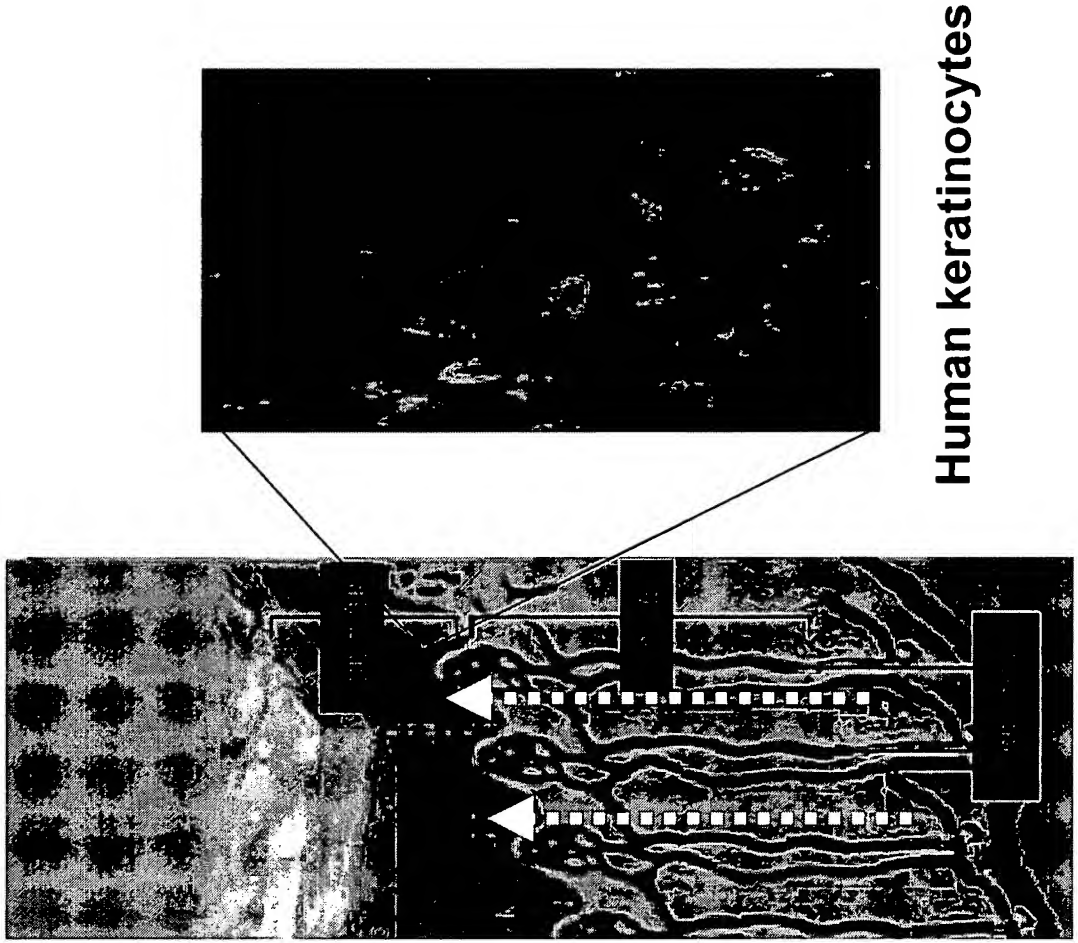


FIG. 10B

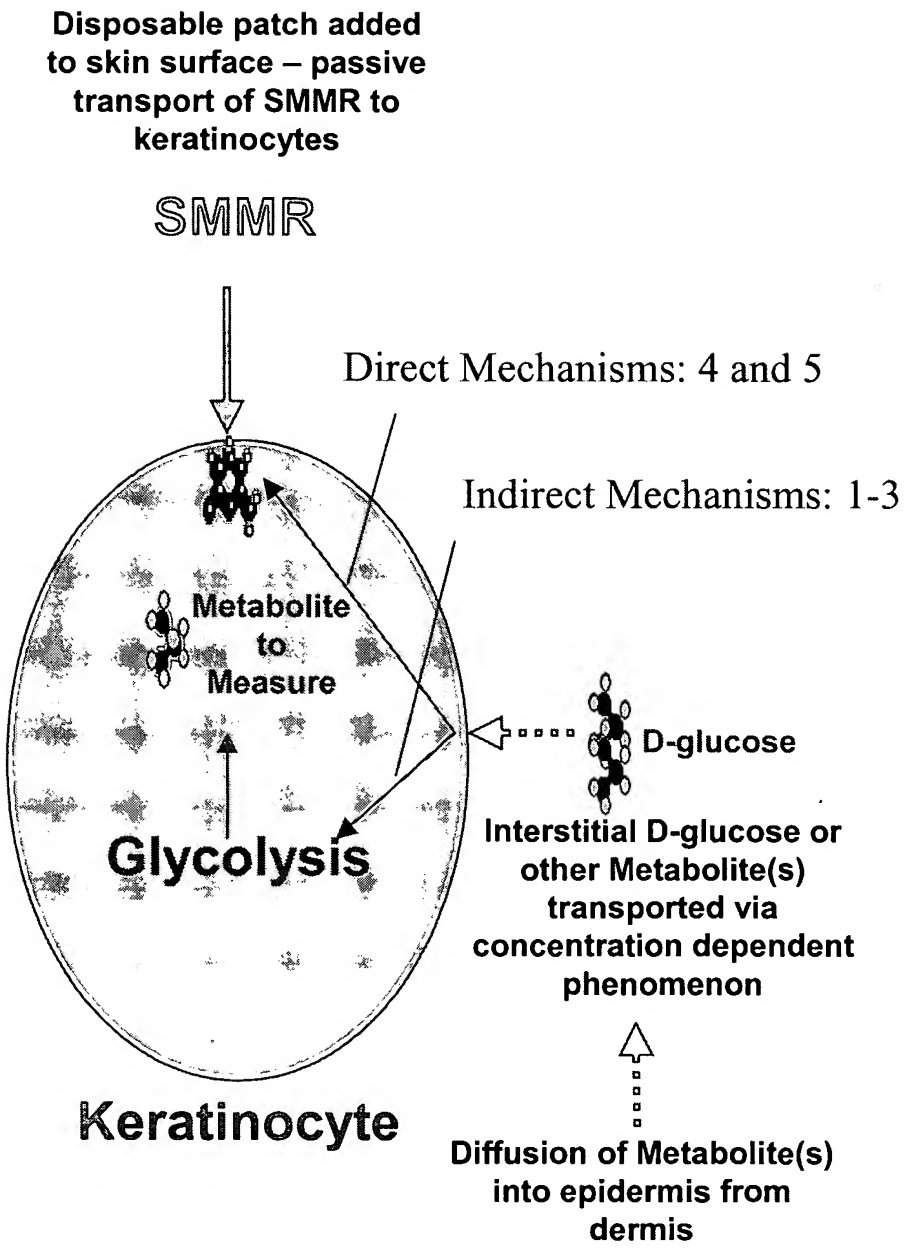

Disposable patch added to skin surface – passive transport of SMMR to keratinocytes
SMMR
Direct Mechanisms: 4 and 5
Indirect Mechanisms: 1-3
Metabolite to Measure
D-glucose
Glycolysis
Interstitial D-glucose or other Metabolite(s) transported via concentration dependent phenomenon
Keratinocyte
Diffusion of Metabolite(s) into epidermis from dermis

FIG. 11

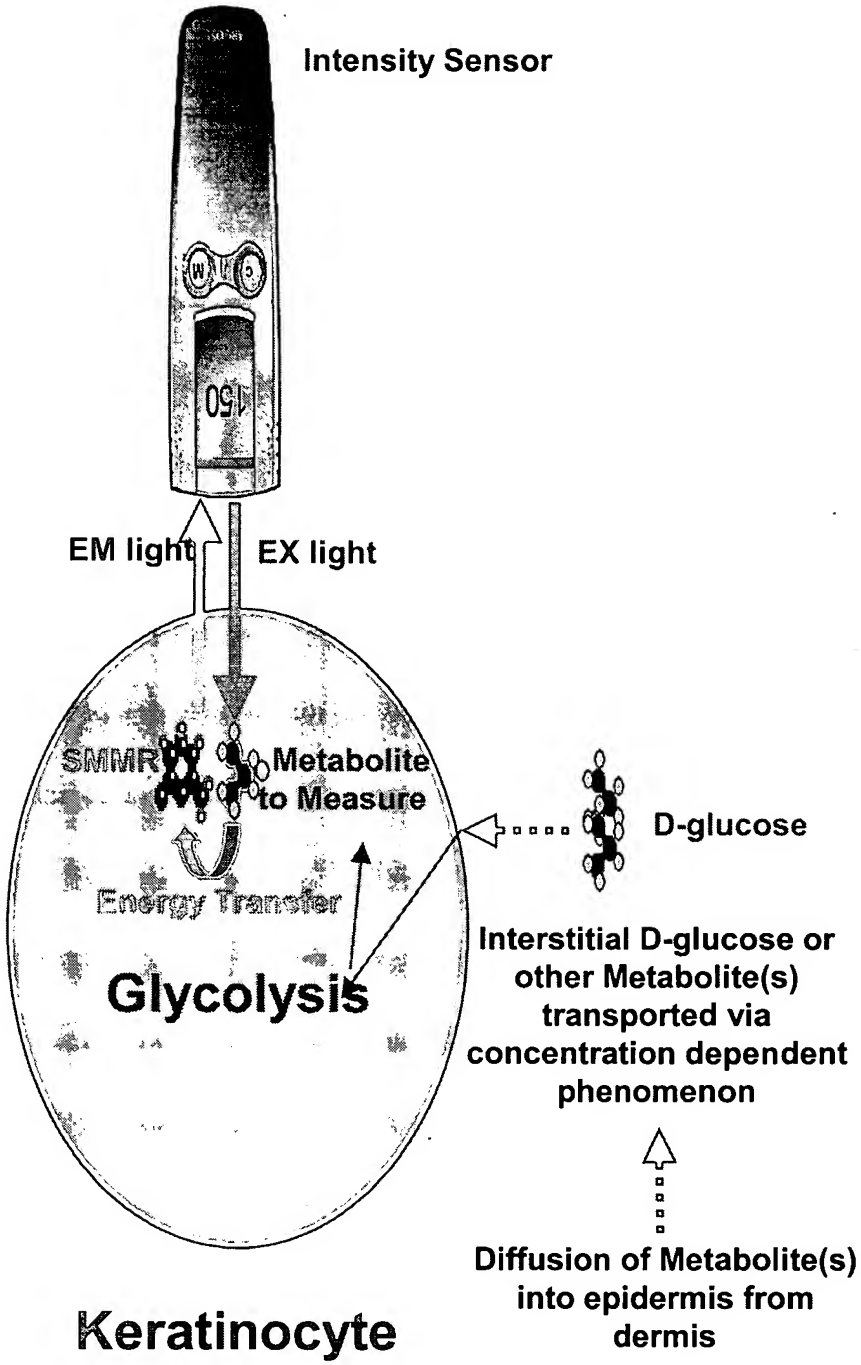

Intensity Sensor
150
EM light
EX light
SMMR
Metabolite to Measure
Energy Transfer
Glycolysis
D-glucose
Interstitial D-glucose or other Metabolite(s) transported via concentration dependent phenomenon
Diffusion of Metabolite(s) into epidermis from dermis
Keratinocyte

FIG. 12

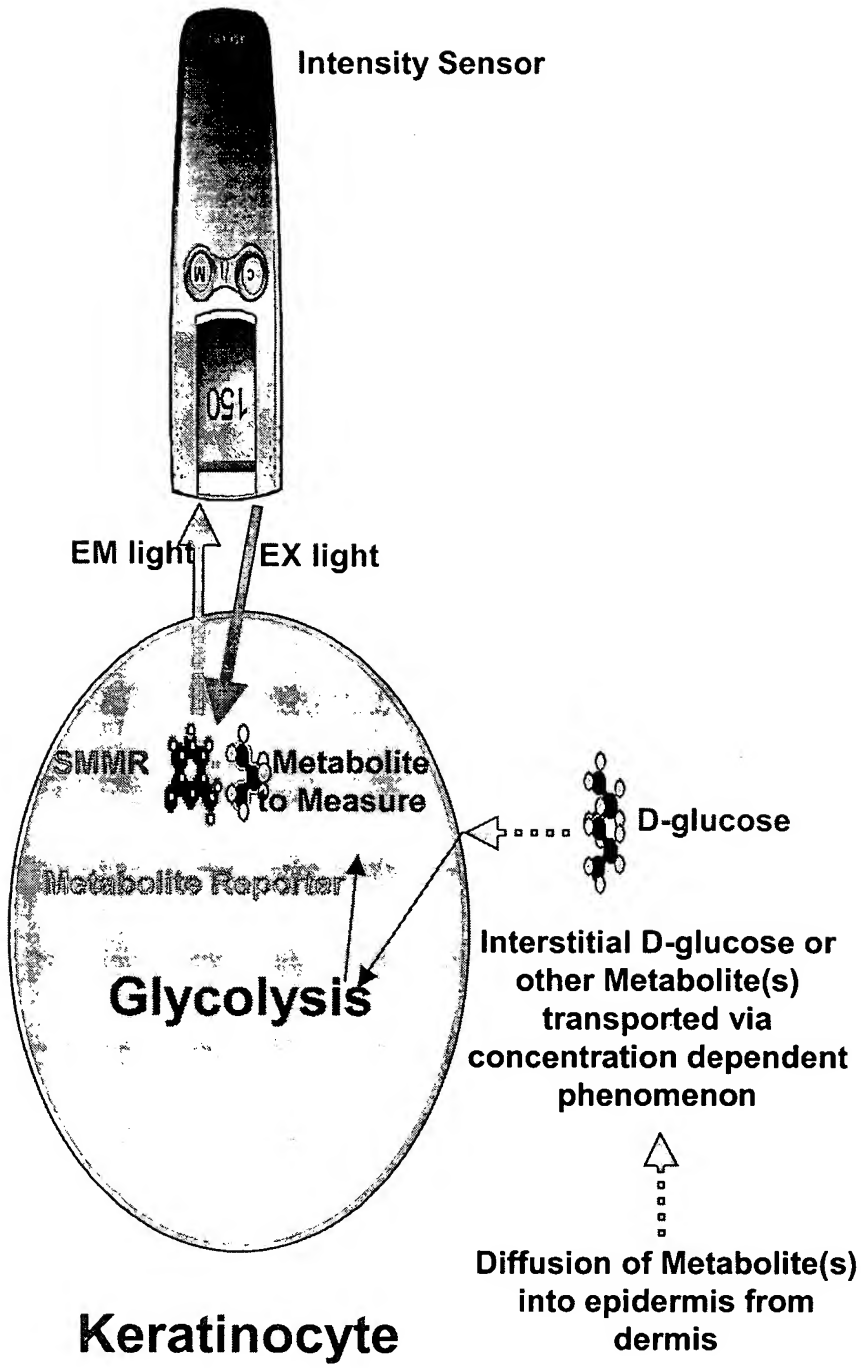

Intensity Sensor
150
EM light
EX light
SMMR
Metabolite to Measure
D-glucose
Metabolite Reporter
Glycolysis
Interstitial D-glucose or other Metabolite(s) transported via concentration dependent phenomenon
Diffusion of Metabolite(s) into epidermis from dermis
Keratinocyte

FIG. 13

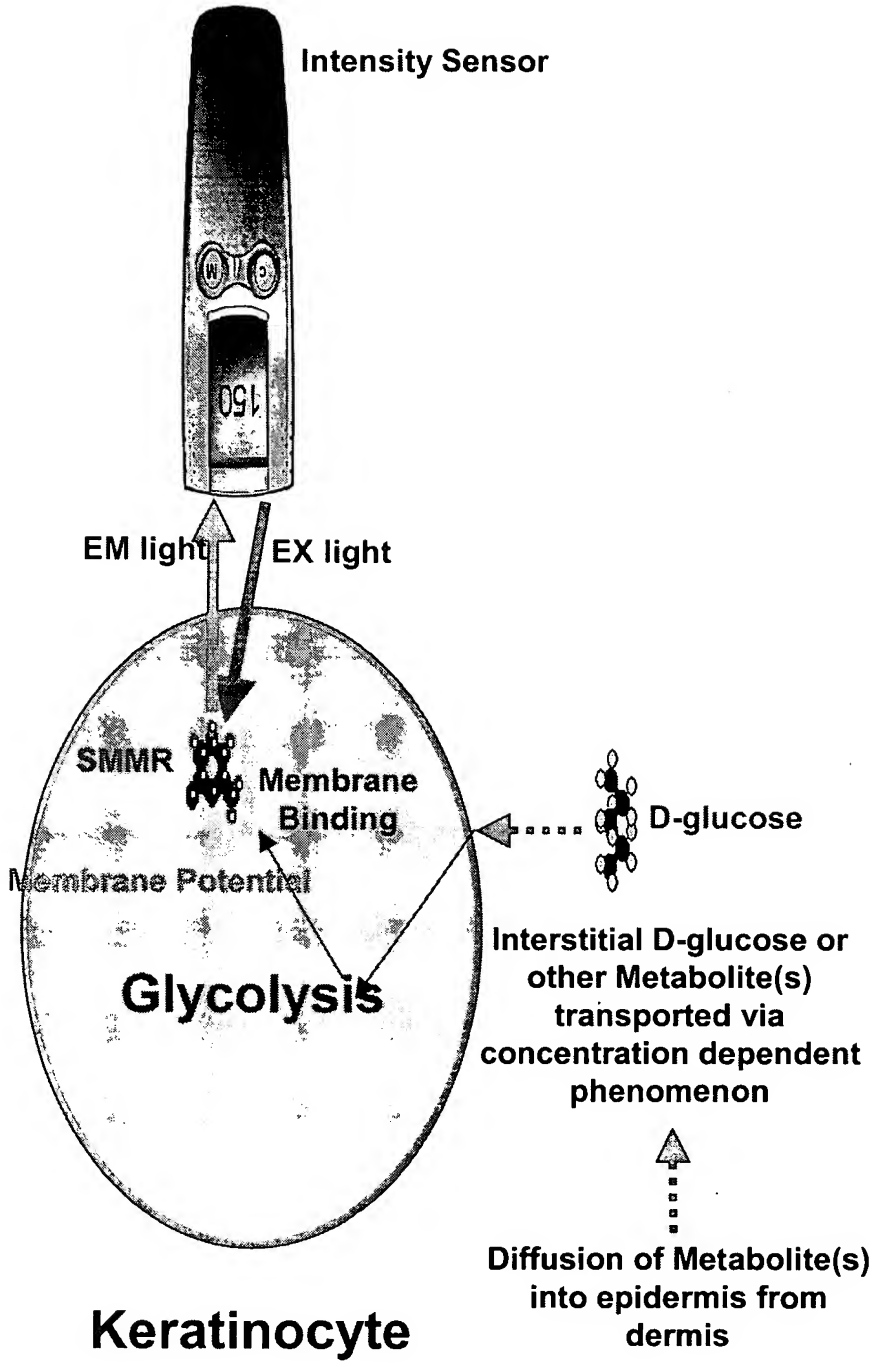

Intensity Sensor
EM light
EX light
SMMR
Membrane Binding
Membrane Potential
D-glucose
Glycolysis
Interstitial D-glucose or other Metabolite(s) transported via concentration dependent phenomenon
Diffusion of Metabolite(s) into epidermis from dermis
Keratinocyte

FIG. 14

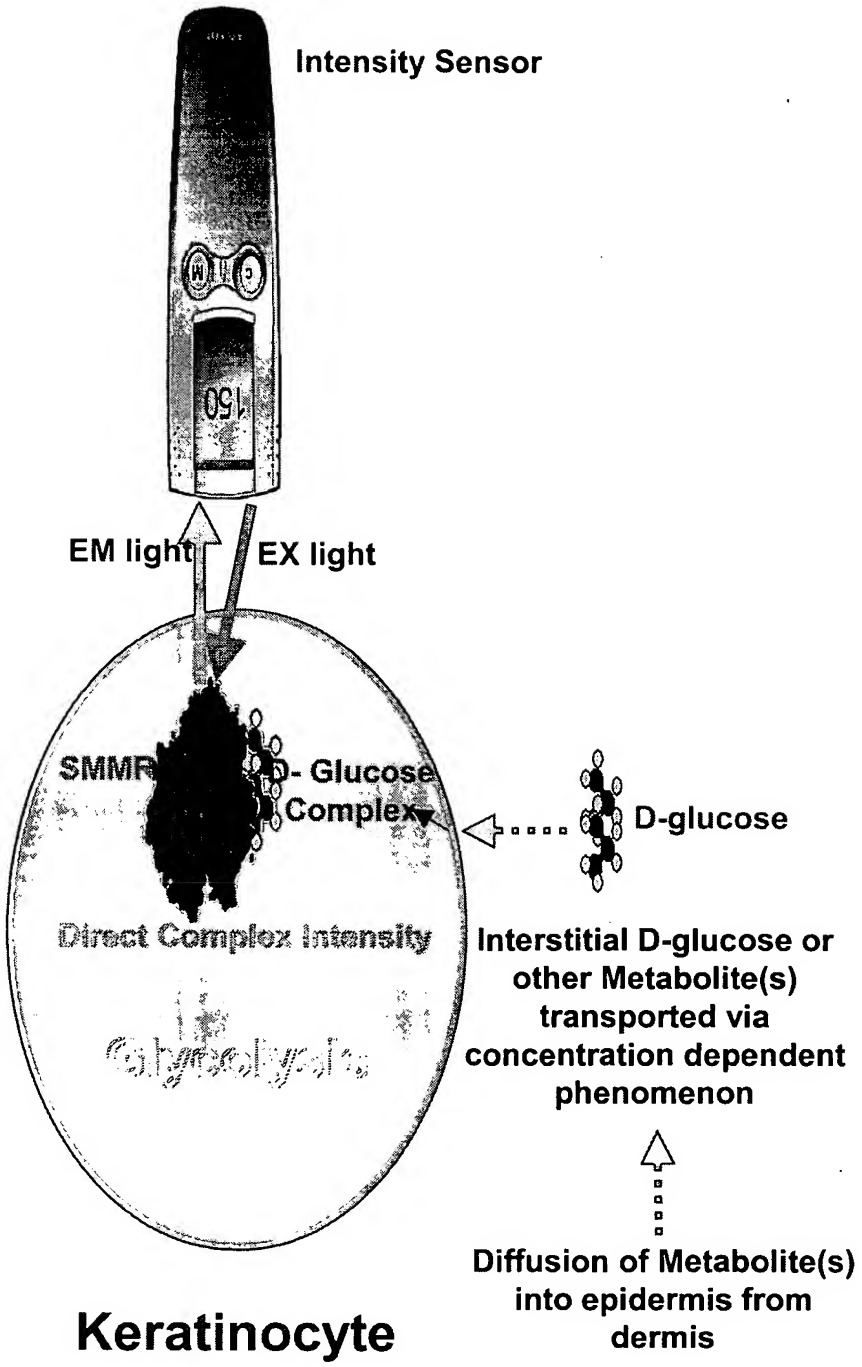

Intensity Sensor
EM light
EX light
SMMR
D- Glucose Complex
D-glucose
Direct Complex Intensity
Interstitial D-glucose or other Metabolite(s) transported via concentration dependent phenomenon
Diffusion of Metabolite(s) into epidermis from dermis
Keratinocyte

FIG. 15

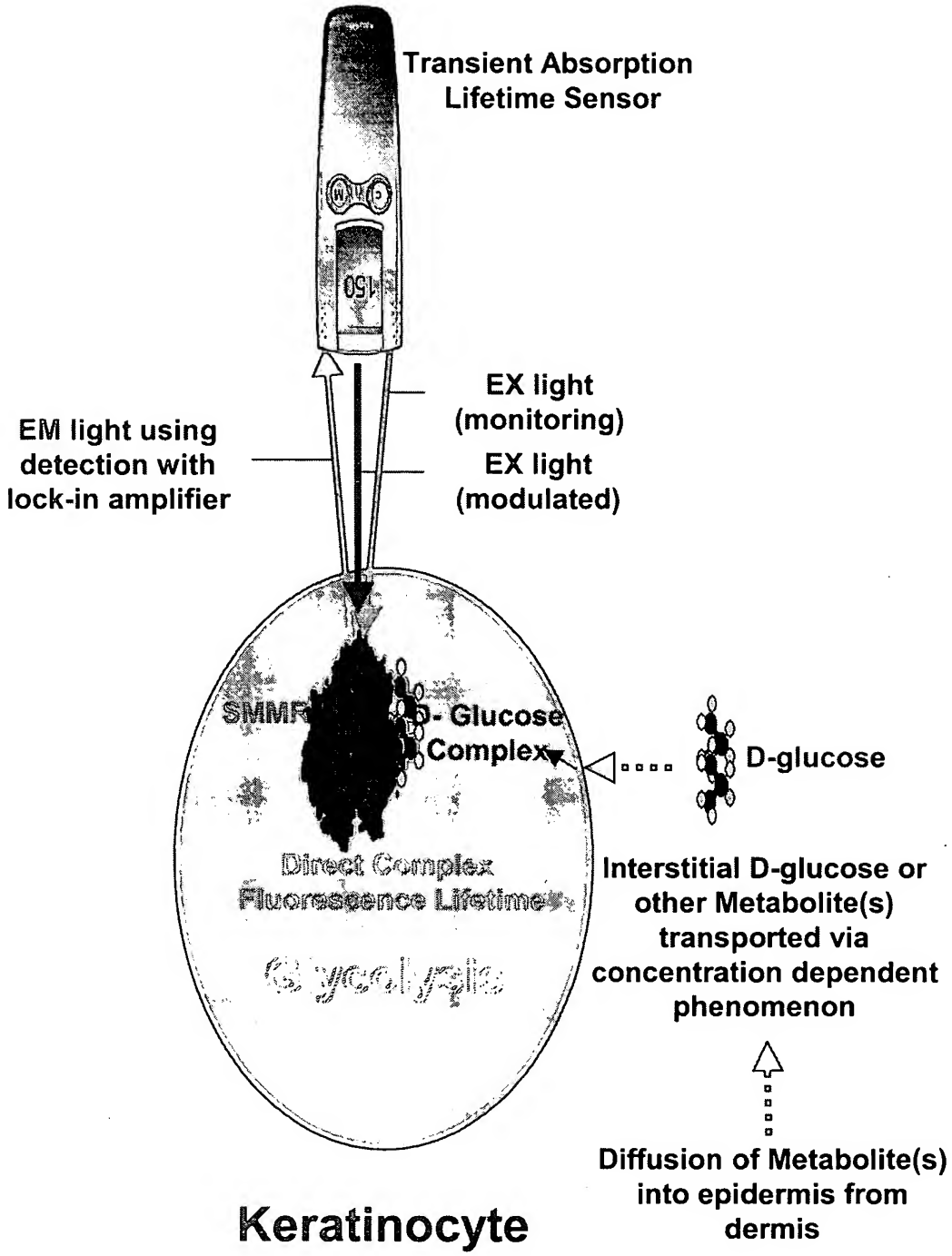

Transient Absorption Lifetime Sensor
EX light (monitoring)
EX light (modulated)
EM light using detection with lock-in amplifier
SMMR
D- Glucose Complex
D-glucose
Direct Complex Fluorescence Lifetime
Interstitial D-glucose or other Metabolite(s) transported via concentration dependent phenomenon
Diffusion of Metabolite(s) into epidermis from dermis
Keratinocyte

FIG. 16

FIG. 17A

Scheme 1

Glc	Intracellular glucose
Pyr	Pyruvate
Glc6-P	Glucose-6-phosphate
Glc1-P	Glucose-1-phosphate
UDP-GLc	UDP-glucose
GluT	Glucose transporter protein

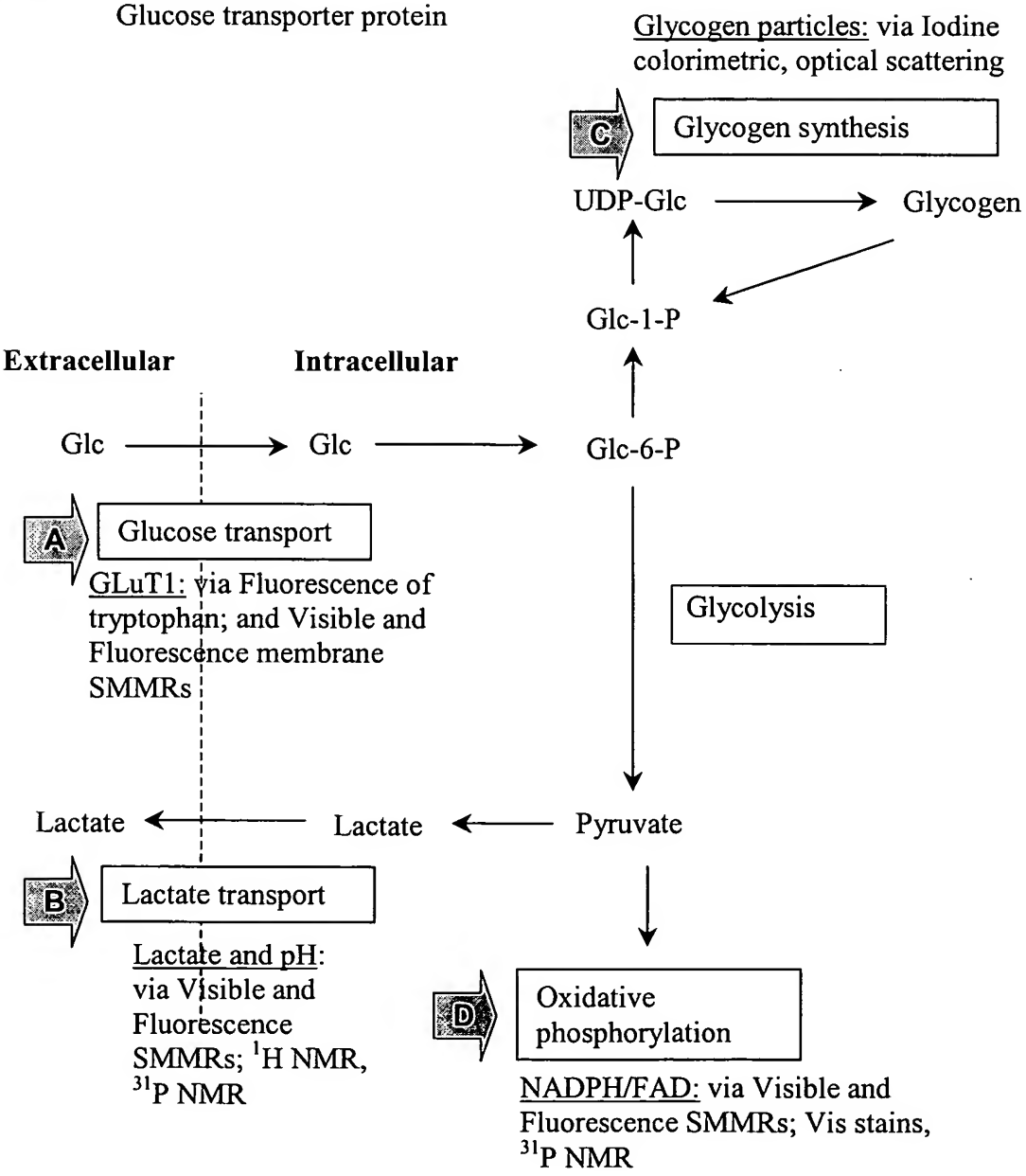


FIG. 17B

Scheme 2. Overview of metabolic pathways for glucose in epidermis

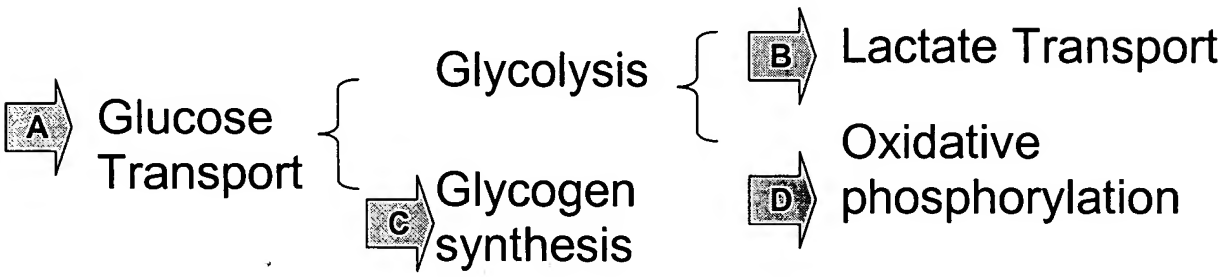
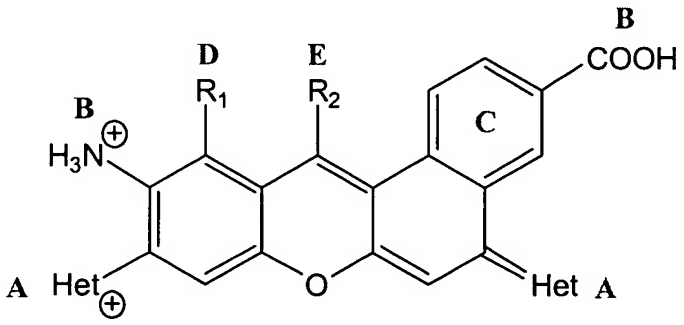


FIG. 17C

Scheme 3. Structure of generic pH sensitive dye for specific action as a lactate/H^+ SMMR



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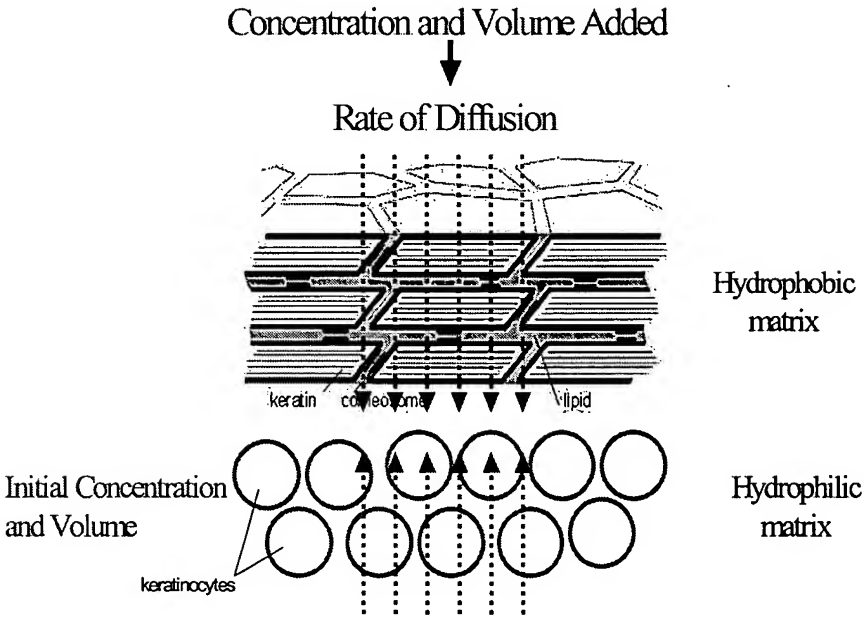


Scheme 4. *In Vivo* Calibration Issues

FIG. 17D

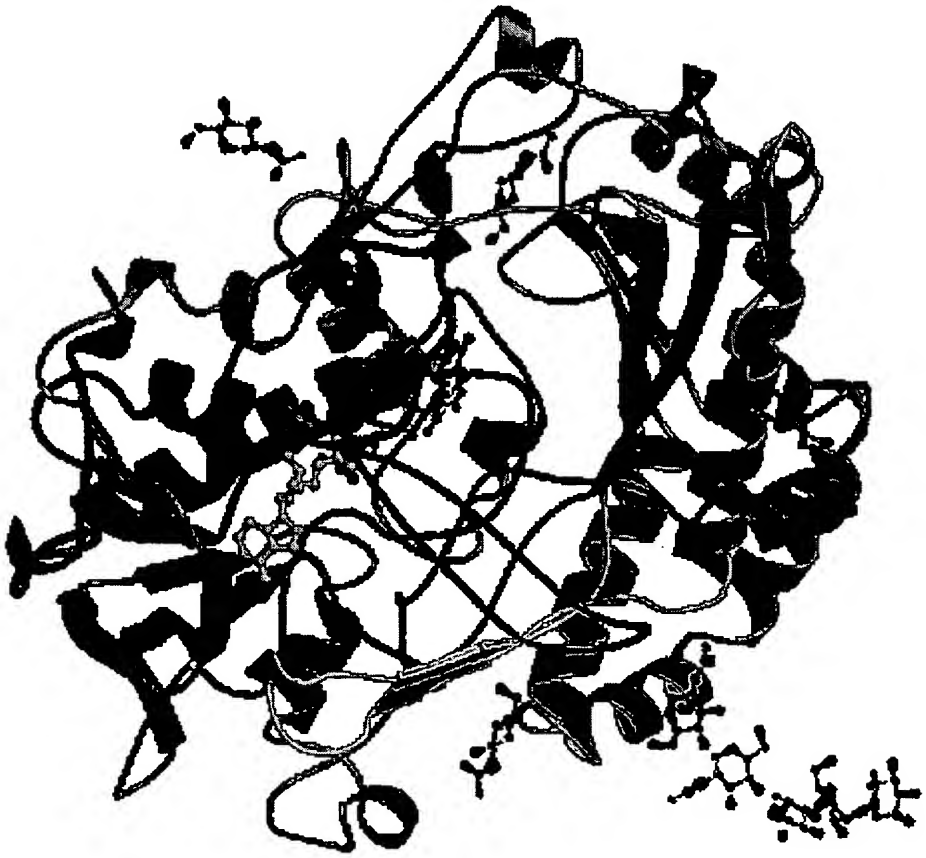


FIG. 18

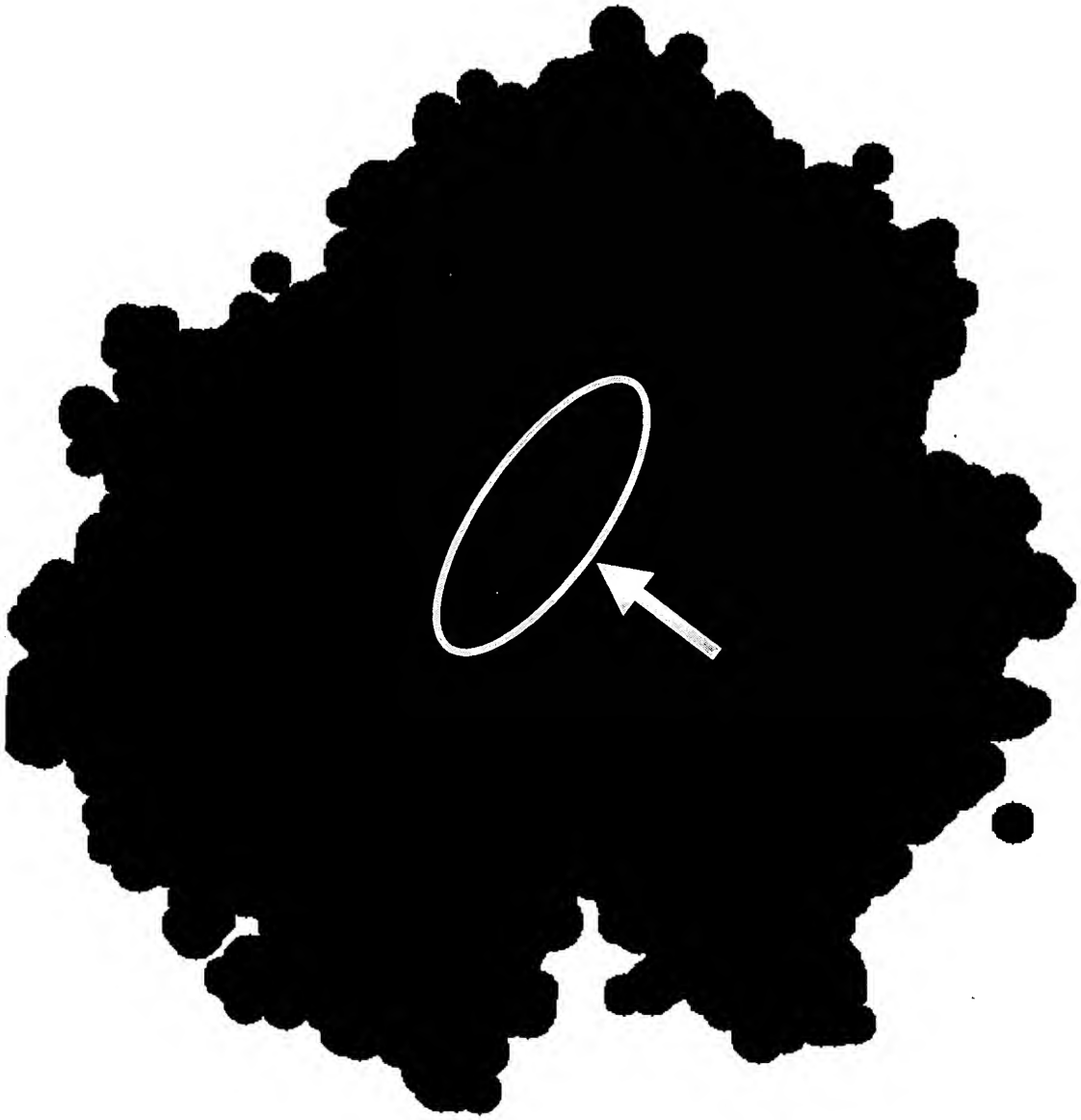


FIG. 19

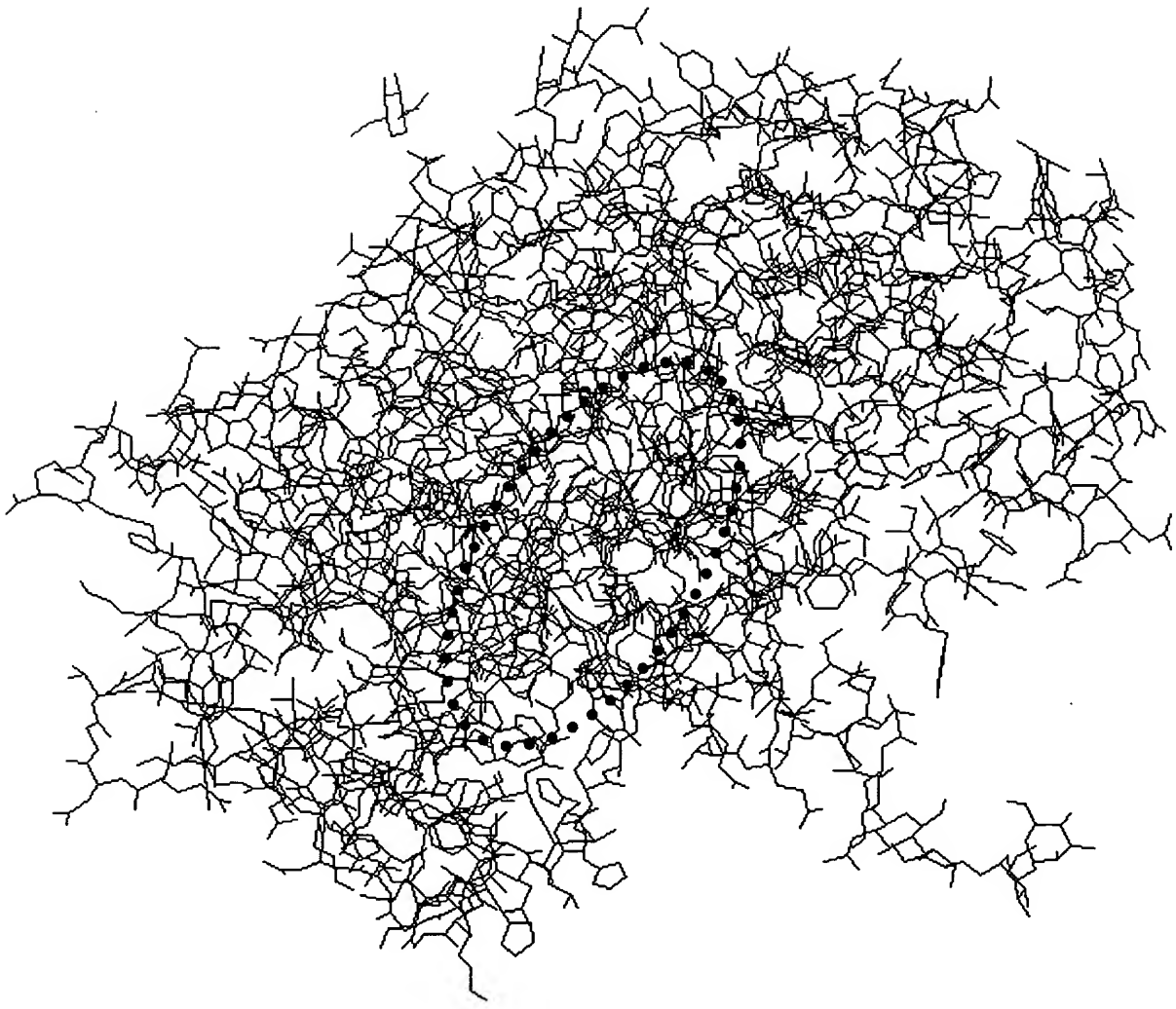


FIG. 20

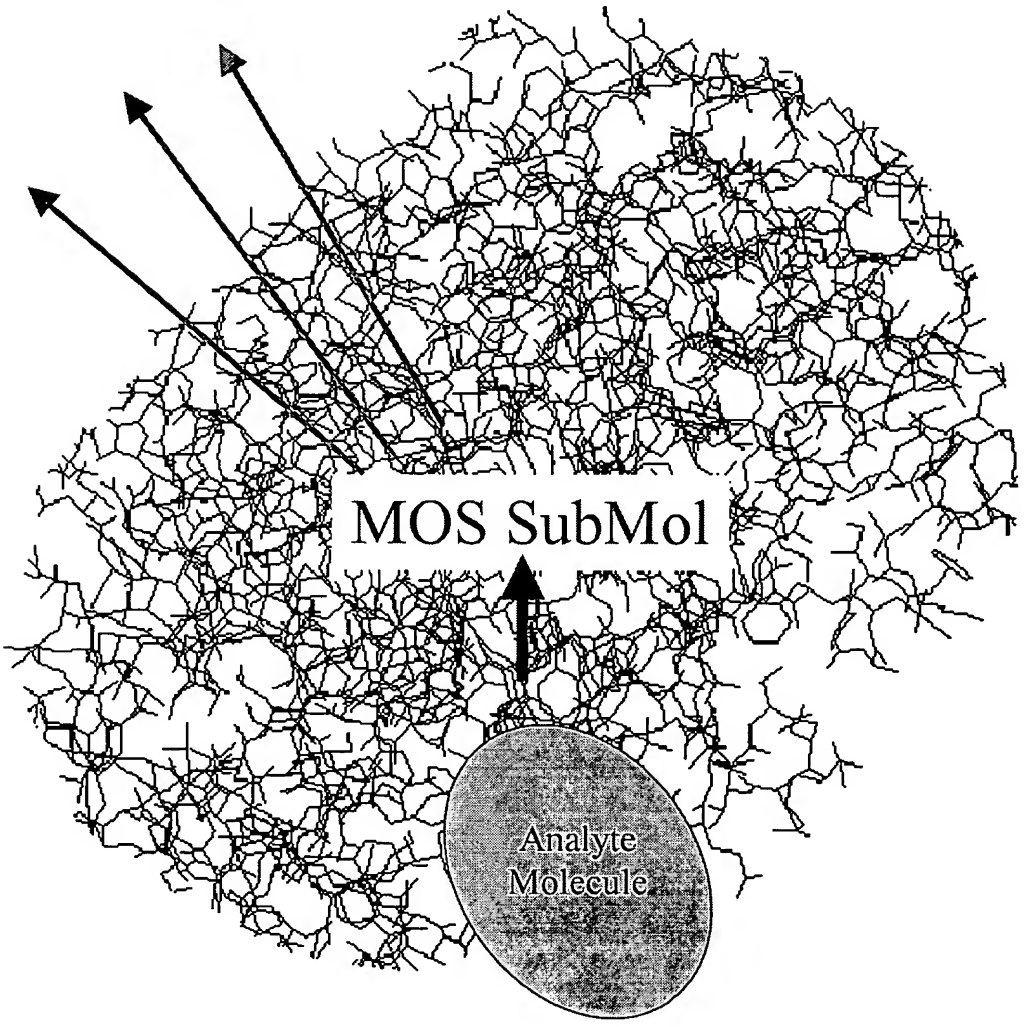


FIG. 21

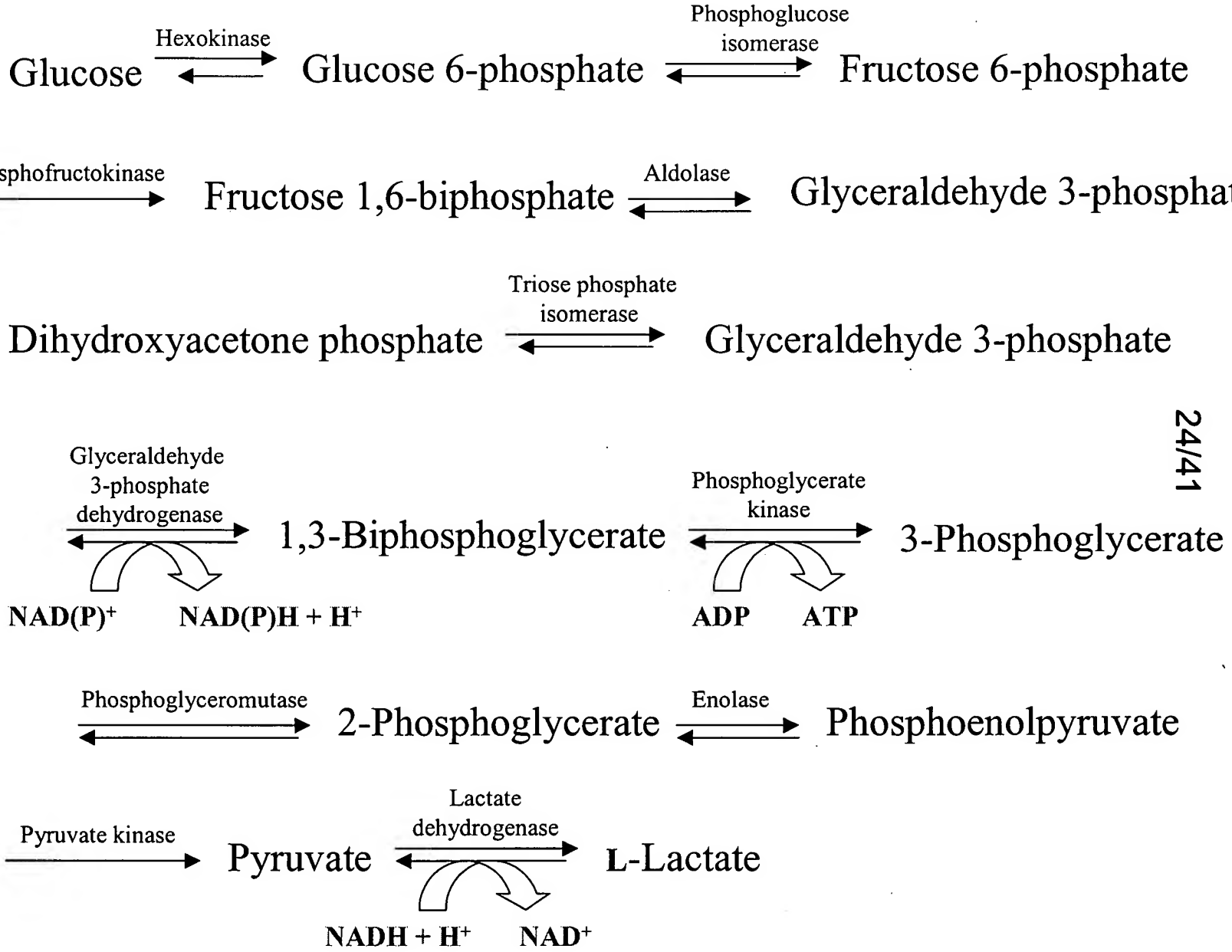
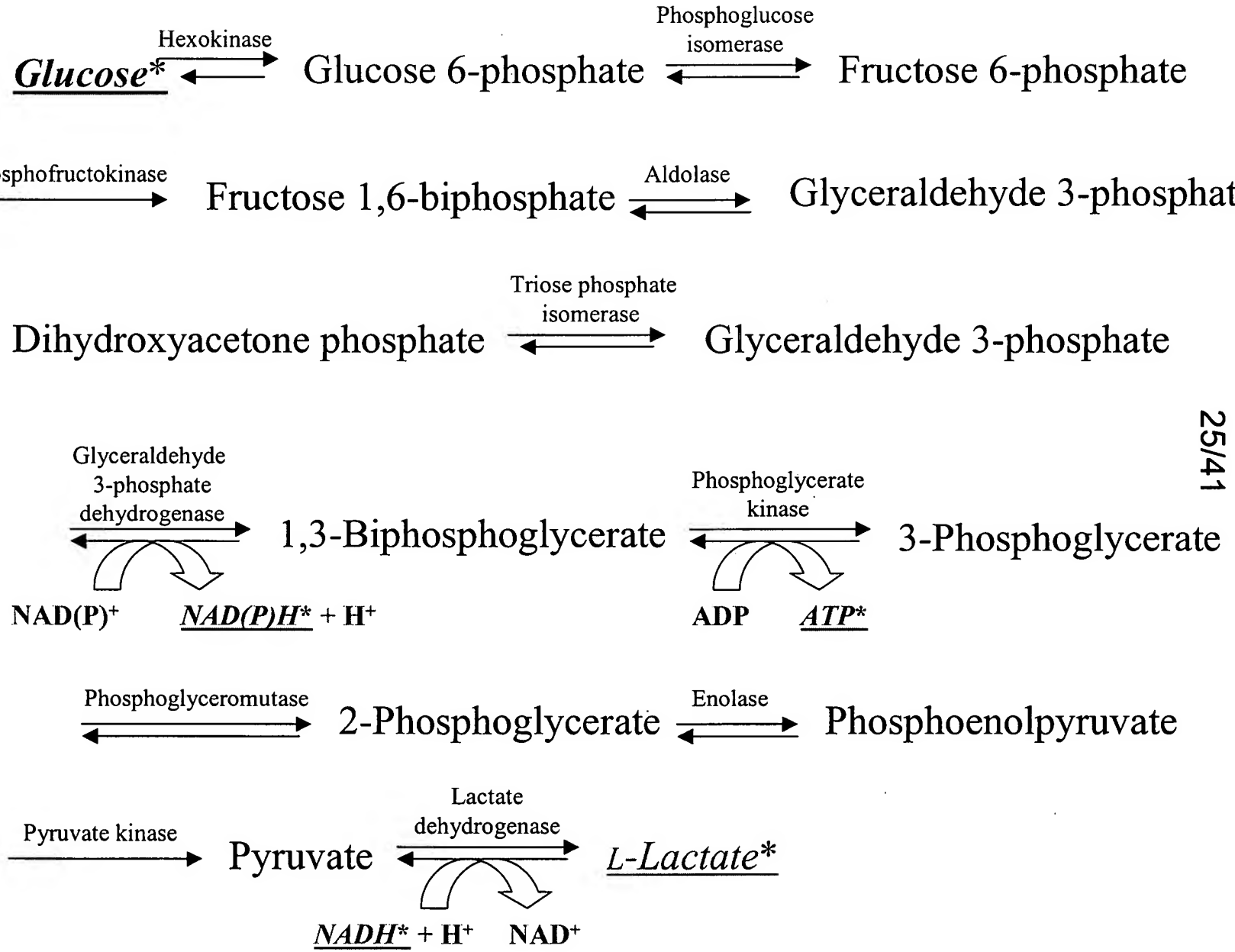


FIG. 22

GLUCOSE GLYCOLYSIS

Sheet 24 of 41
Title: Non-invasive measurement of analytes
Inventors: Workman, Lambert and Coleman
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000
Filed: HEREWITH

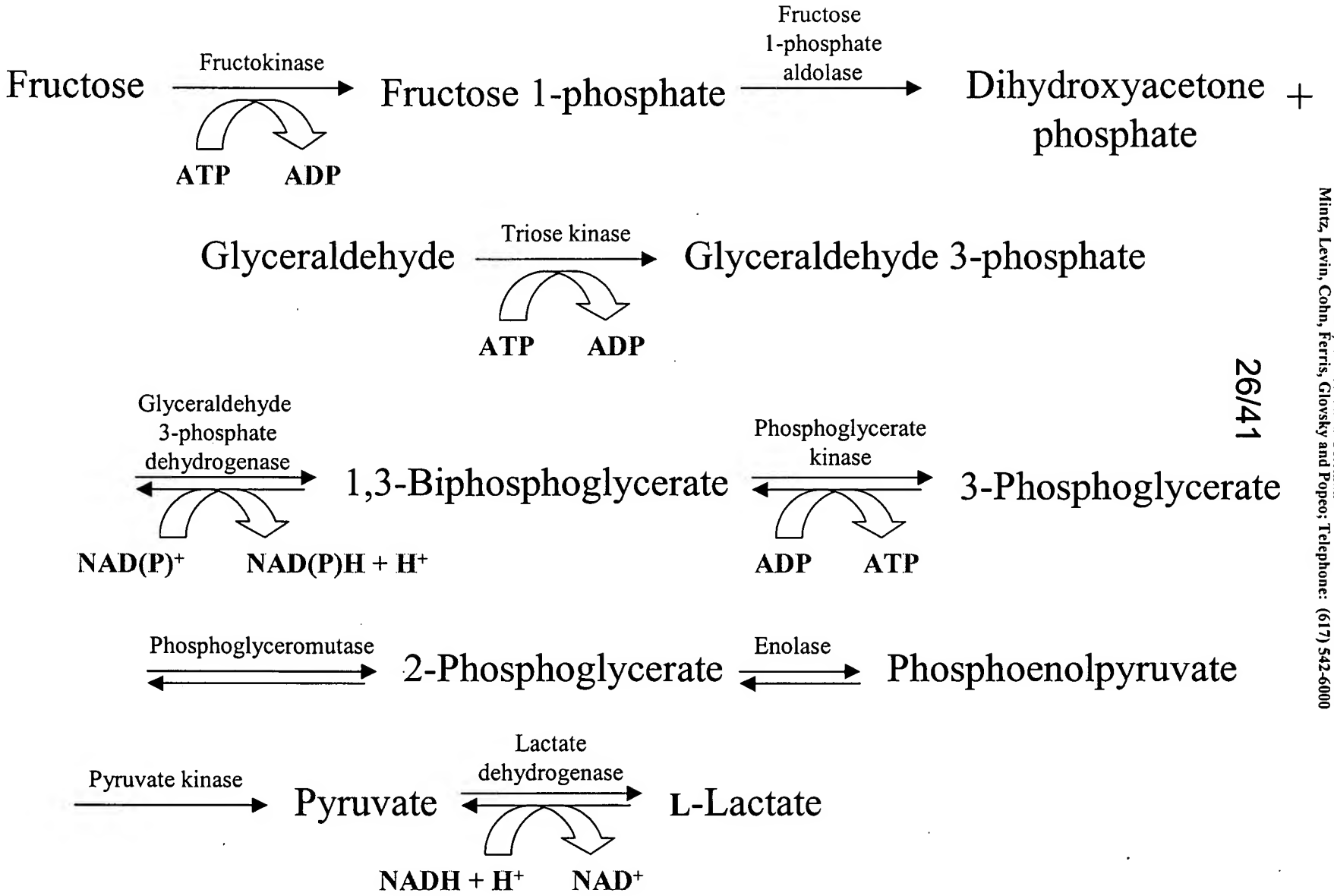


***Detectable Analytes (direct or indirect)**

FIG. 23

GLUCOSE GLYCOLYSIS

Sheet 25 of 41
Title: Non-invasive measurement of analytes
Inventors: Workman, Lambert and Coleman
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000
Filed: HEREWITH



FRUCTOSE GLYCOLYSIS

FIG. 24

Sheet 26 of 41
Title: Non-invasive measurement of analytes
Inventors: Workman, Lambert and Coleman
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000
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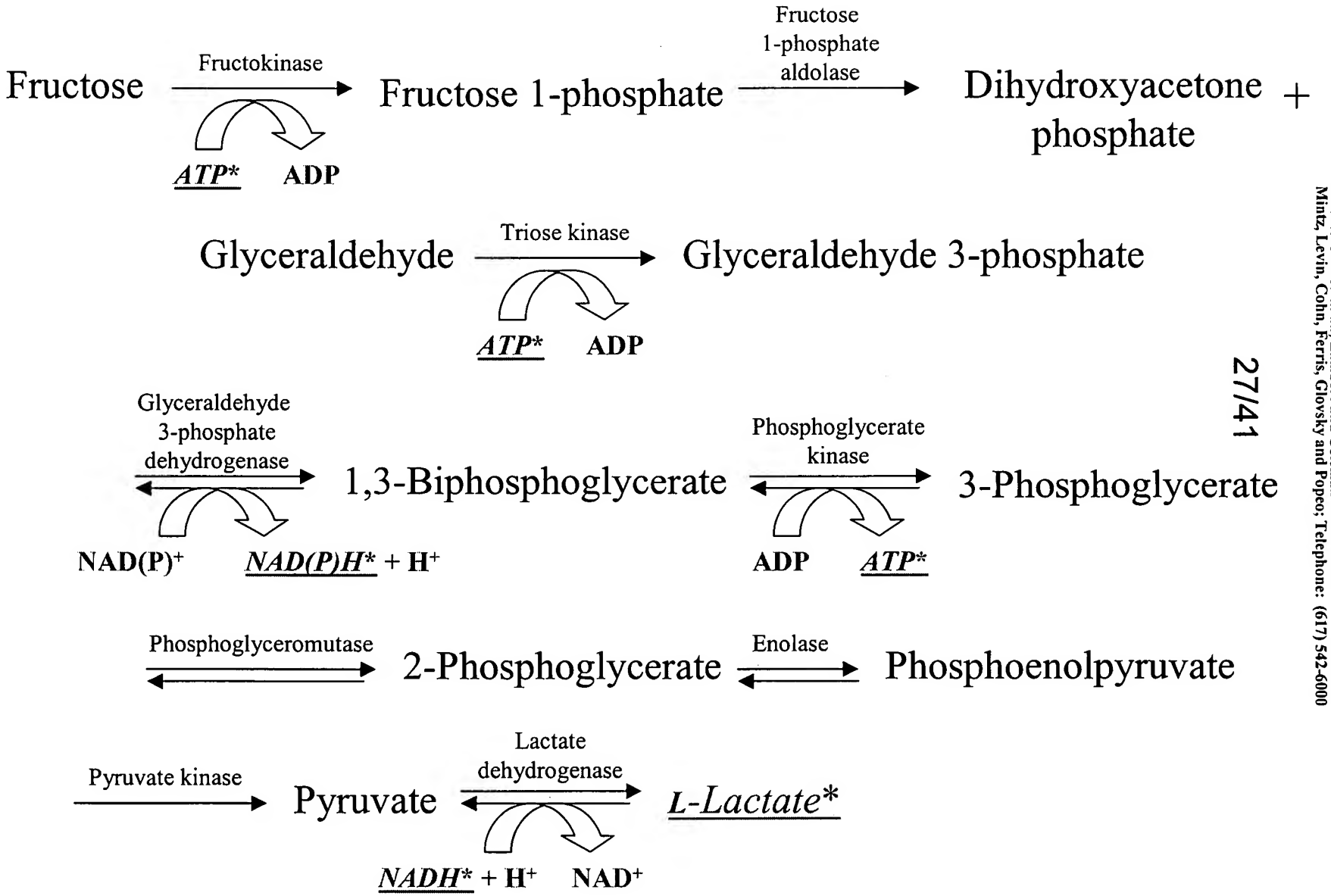

Detectable Analytes (direct or indirect)

FIG. 25

FRUCTOSE GLYCOLYSIS

Sheet 27 of 41
Title: Non-invasive measurement of analytes
Inventors: Workman, Lambert and Coleman
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000
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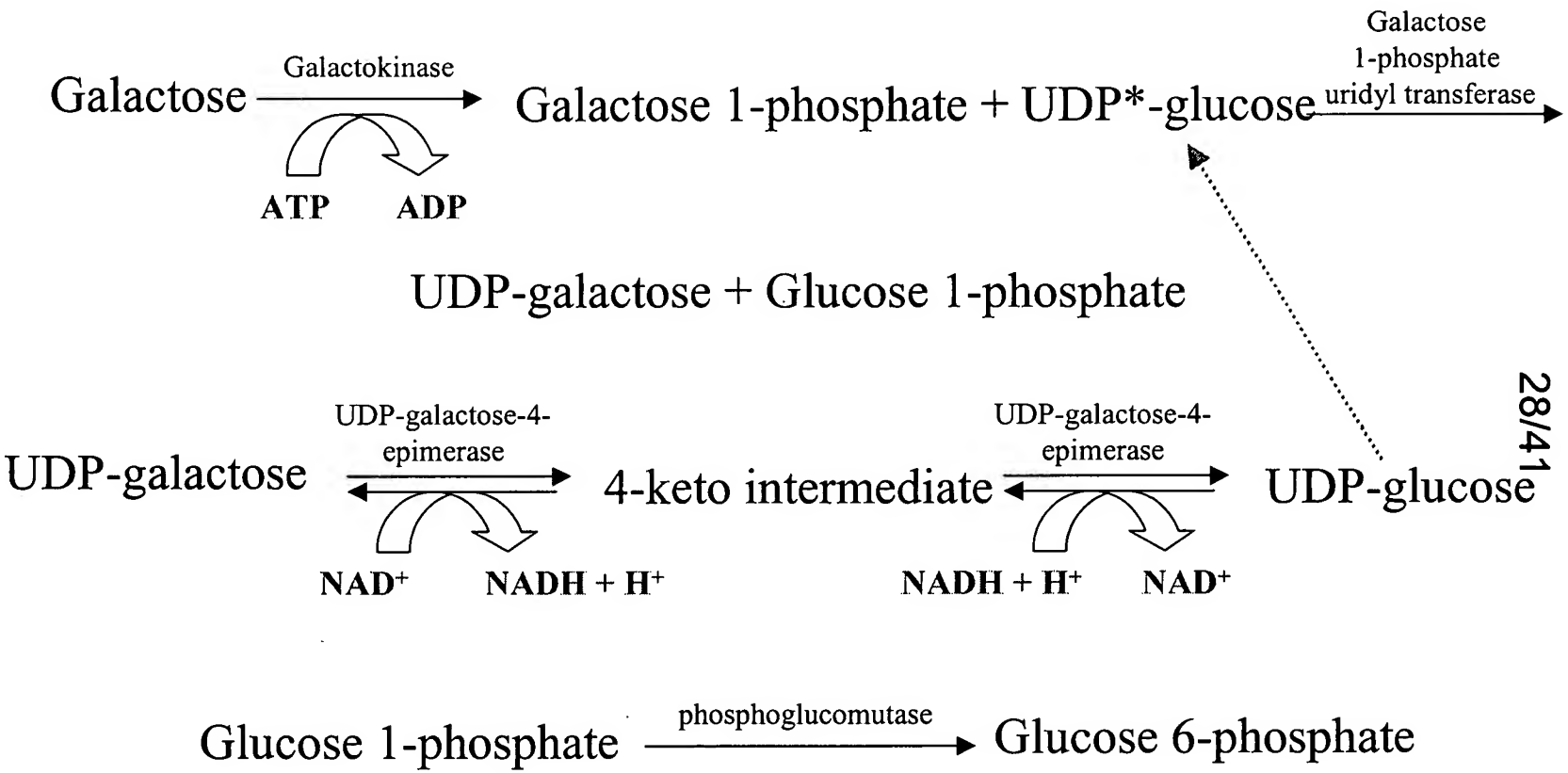

Continuation of Glycolysis

*uridine diphosphate (UDP)

GALACTOSE GLYCOLYSIS

FIG. 26

Sheet 28 of 41
Title: Non-Invasive measurement of analytes
Inventors: Workman, Lambert and Coleman
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000
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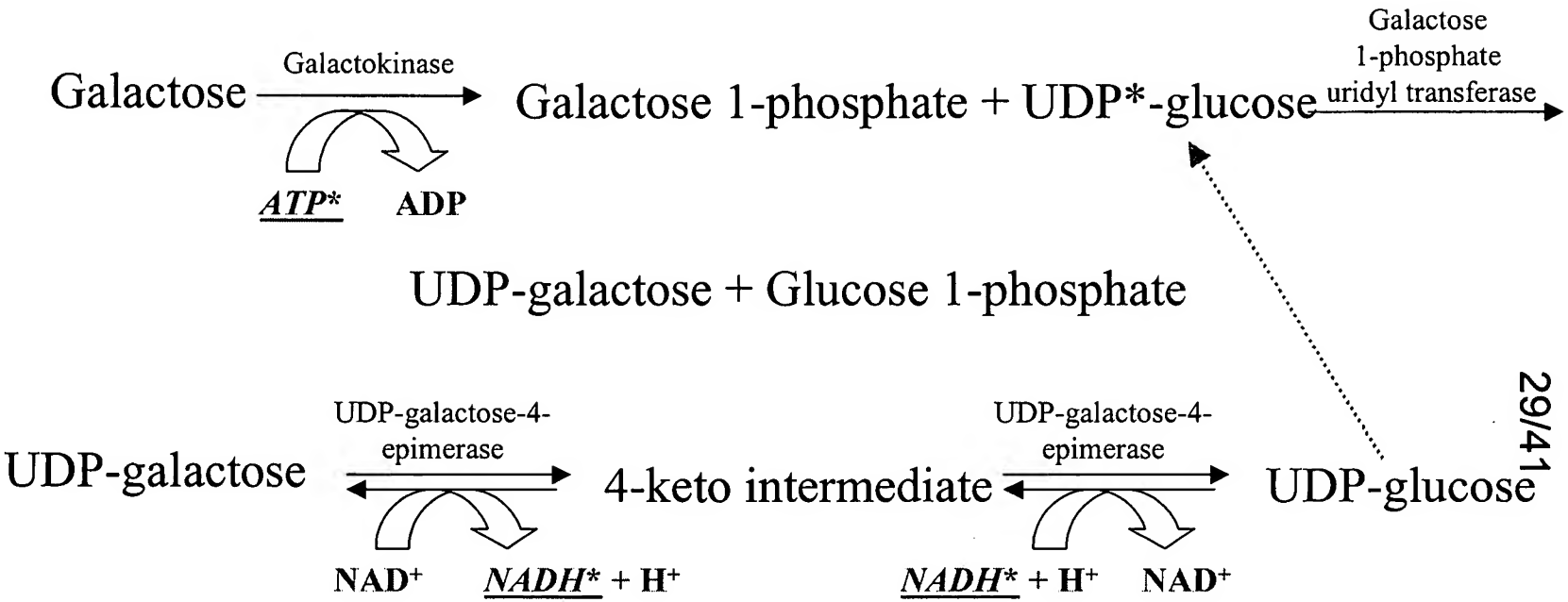

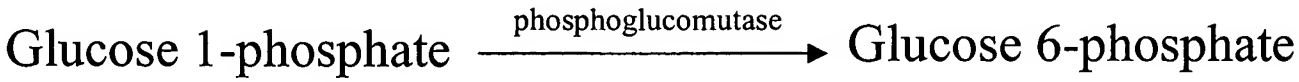

Continuation of Glycolysis

*uridine diphosphate (UDP)

***Detectable Analytes (direct or indirect)**

FIG. 27

GALACTOSE GLYCOLYSIS

Sheet 29 of 41
Title: Non-invasive measurement of analytes
Inventors: Workman, Lambert and Coleman
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000
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SMMR Mechanisms of Signal

1.0 Enhancement of Signal-to-noise of native autofluorescence

- 1.1 Energy Transfer from NADH, NAD(P)H, or FAD to Reporters (boosts signal by 5 to 50) indicating redox transfer coenzyme activity within cells and tissues
- 1.2 Redox potential Reporters indicates number of mitochondrial transmembrane redox potential events

2.0 Enhancement of Specific Metabolite and Precursor Signals

- 2.1 Lactate Reporters indicate lactate formation from anaerobic glycolysis activity
- 2.2 Ca^{2+} Reporters indicate available ATP and ion pump transport activity fueled by glycolytic activity

3.0 Direct Glucose Reporters indicating quantitative levels of d-glucose

- 3.1 Protein-labeled fluorophores
- 3.2 proteins with a photooxidizable cofactor (such as FAD) to observe $^3FAD^*$

Sheet 30 of 41
Title: Non-invasive measurement of analytes
Inventors: Workman, Lambert and Coleman
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000
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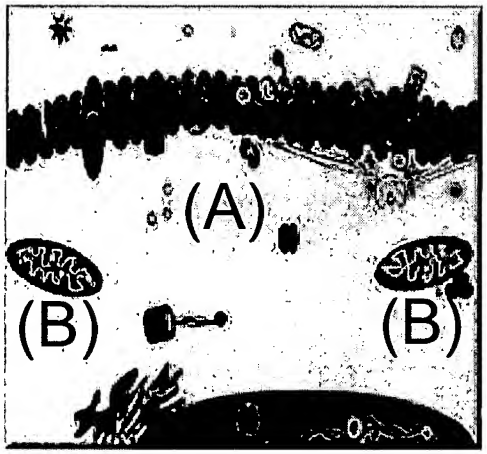
FIG. 28

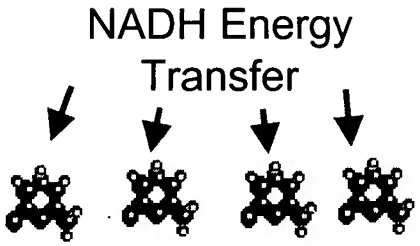
Energy Transfer Reporters

Glycolysis within the cell Cytosol (A)

$$\text{Glucose} + 2\,P_i + 2\text{ADP}\ 2\text{NAD}^+ \rightarrow 2\text{Pyruvate} + 2\,\text{NADH} + 2\,\text{ATP} + 2\text{H}^+ + 2\text{H}_2\text{O}$$



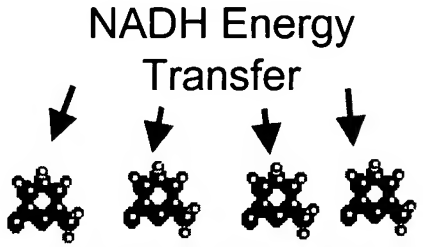
Cell Cross-section



Small Molecule Metabolic Reporters (SMMR)

Within the Mitochondria (B)

$$\text{NAD}^+ + \text{Pyruvate} + \text{CoA} \rightarrow \text{Acetyl CoA} + \text{CO}_2 + \text{NADH}$$



Small Molecule Metabolic Reporters (SMMR)

Sheet 31 of 41
Title: Non-Invasive measurement of analytes
Inventors: Workman, Lambert and Coleman
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000
Filed: HEREWITH

FIG. 29

Redox Potential Reporters

In the Mitochondria

$NAD^+ + Pyruvate + CoA \rightarrow Acetyl\ CoA + CO_2 + NADH + H^+$

Increase in glucose concentration increases the mitochondrial membrane potential causing more small molecule metabolic reporter (SMMR) units to attach to the membrane. This causes fluorescence quenching proportional to changes in glucose concentration

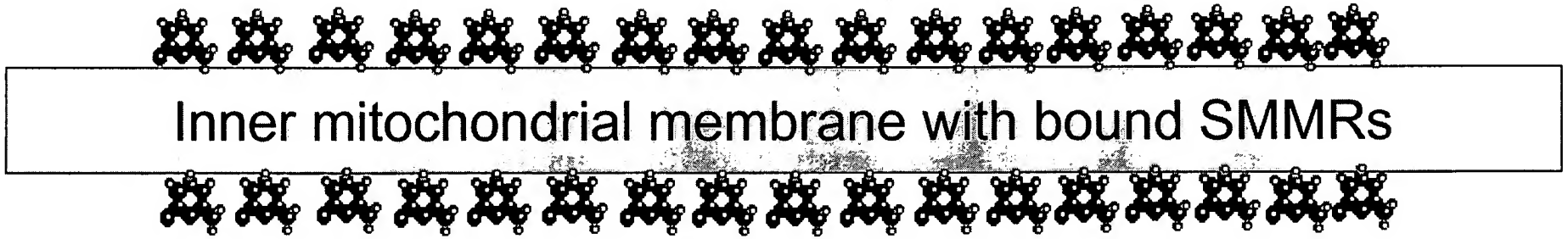


FIG. 30

Sheet 32 of 41
Title: Non-invasive measurement of analytes
Inventors: Workman, Lambert and Coleman
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000
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Lactate Reporters

Anaerobic Glycolysis

$$\text{Glucose} + 2P_i + 2ADP \rightarrow 2 \text{ lactate} + 2ATP + 2H_2O$$

Increase in glucose concentration increases the lactate formation in a 2:1 ratio. A small molecule metabolic reporter (SMMR) is used to detect pH changes caused by lactate concentration. The pH changes are directly related to glucose concentration

pH change reduces FL of SMMRs

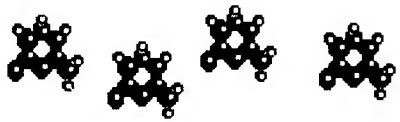

FIG. 31

Ca^{2+} Reporters

Ca^{2+} and ATPase

Cell signaling is accomplished using ions such as Ca^{2+}. When the cell performs a signaling action Ca^{2+} is released from ion storage into the cytosol where it triggers cellular activities. A small molecule metabolic reporter (SMMR) is used to detect Ca^{2+} changes caused by changes in ion concentration within the cytosol. The Ca^{2+} concentration changes are directly related to healthy cell function. After signaling the Ca^{2+} is pumped back into storage using ATPase synthesized from Available ATP. Each molecule of ATP pumps 2 Ca^{2+}. If the ion pumps are not working due to respiratory stress the ion concentrations equilibrate by diffusion since the pumps are incapacitated. The ion concentration gradients are maintained by ATP regulated pumps.

Ca^{2+} changes increase FL of SMMRs

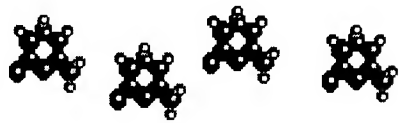

FIG. 32

Sheet 34 of 41
Title: 3on-invasive measurement of analytes
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O_2 Reporters

Aerobic Respiration

$$\text{Glucose} + 6O_2 + 36\ \text{ADP} + 36P_i \rightarrow 6CO_2 + 6H_2O + 36\ \text{ATP} + \text{Heat}$$

Increase in molecular oxygen indicates a favorable environment for aerobic respiration. A small molecule metabolic reporter (SMMR) is used to detect O_2 changes in the cellular environment. The O_2 changes are directly related to ability to manufacture ATP.

O_2 changes increase FL of SMMRs

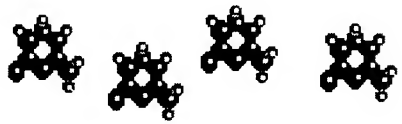

FIG. 33

Sheet 35 of 41
Title: Non-invasive measurement of analytes
Inventors: Workman, Lambert and Coleman
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000
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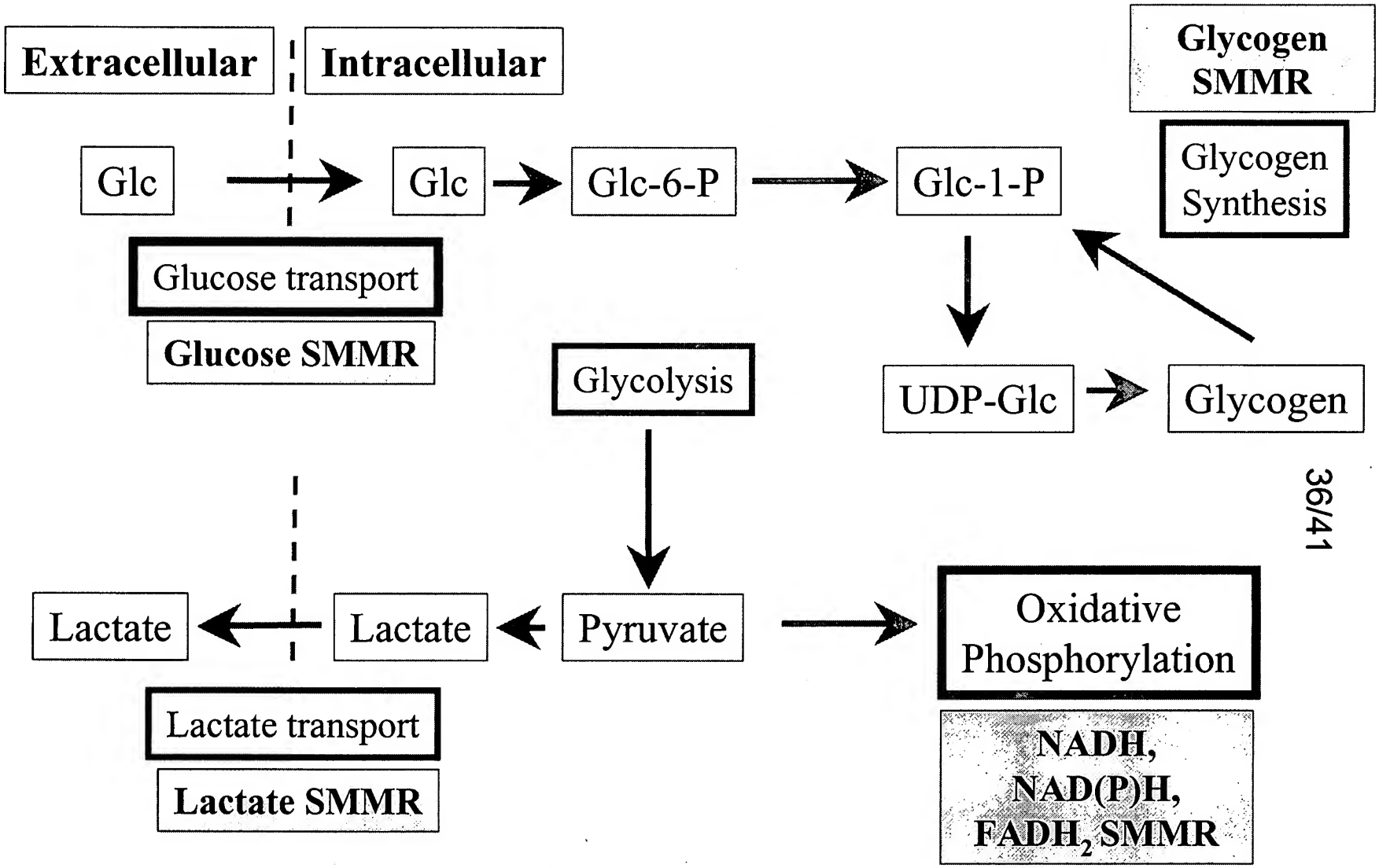

SMMR has been used to establish analytical methods for measuring each glucose pathway for a variety of cell types

FIG. 34

SMMR Application Summary

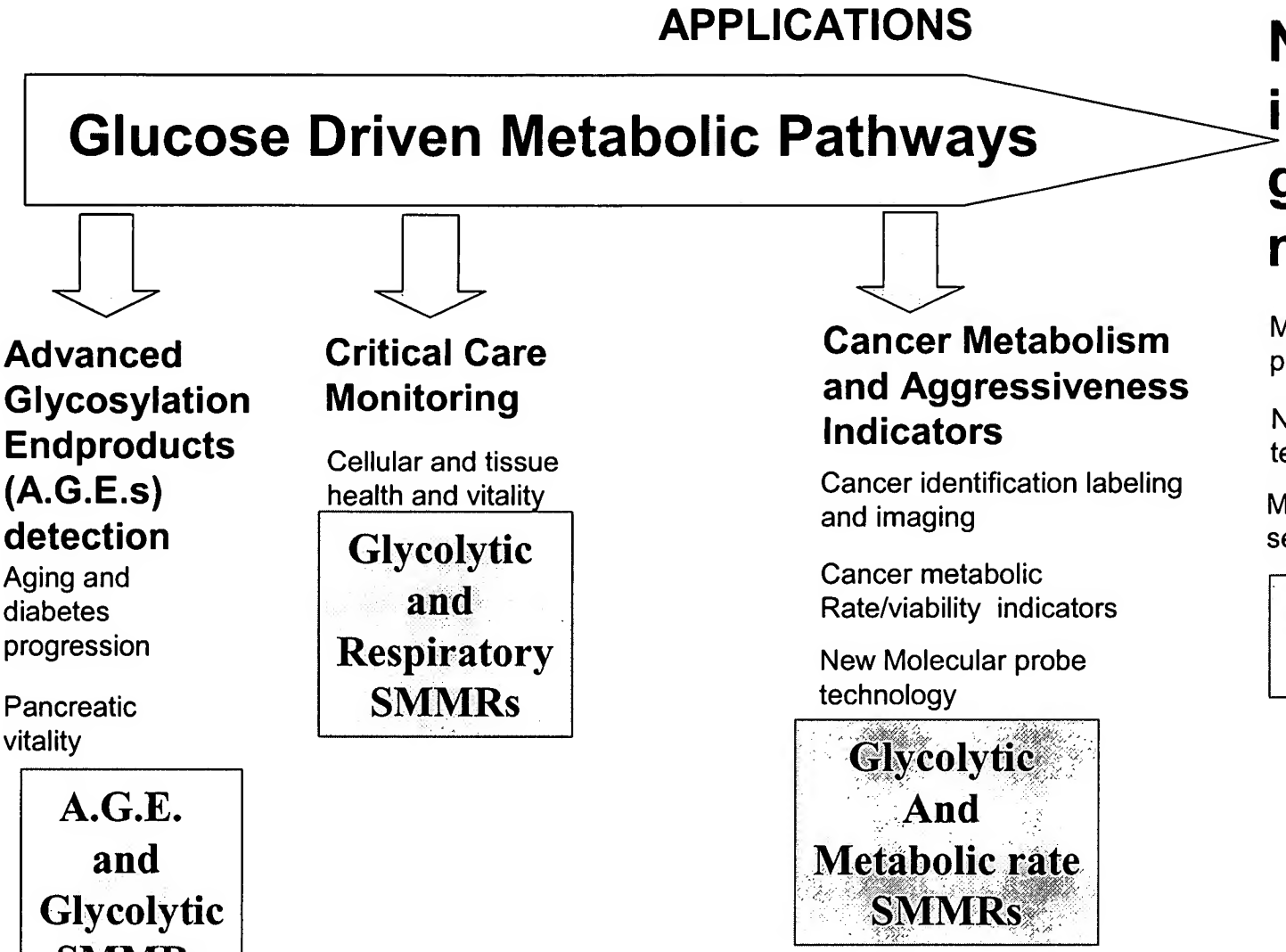

FIG. 35

Sheet 37 of 41
Title: Non-invasive measurement of analytes
Inventors: Workman, Lambert and Coleman
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000
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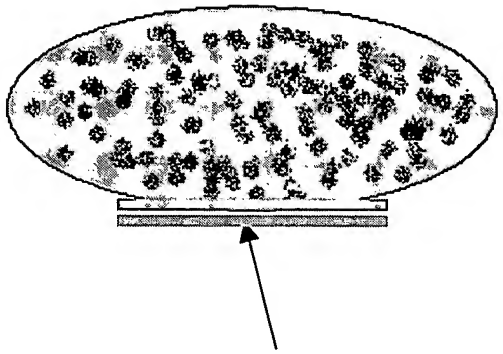
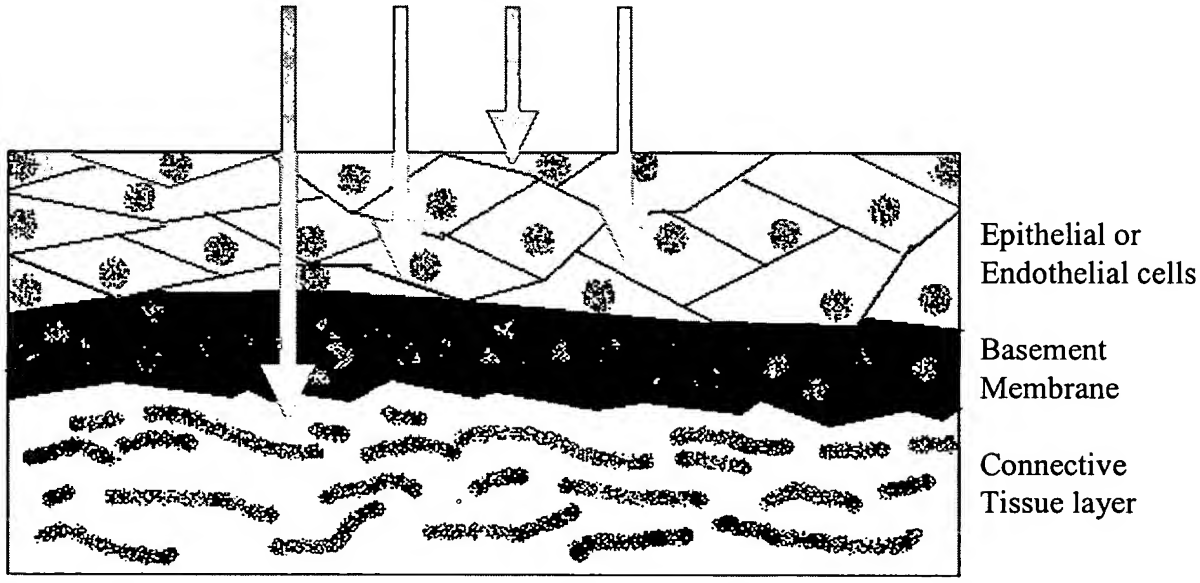
Method for adding SMMR to peripheral epithelial cells in tissues and organs



A. SMMRs are applied to tissue surface

B. SMMRs are transported for up to 10-300 microns into the top of the tissue using passive or active transport



Outer (or inner) membrane of tissues and organs

FIG. 36

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SMMR for Metabolite Discrimination or Imaging

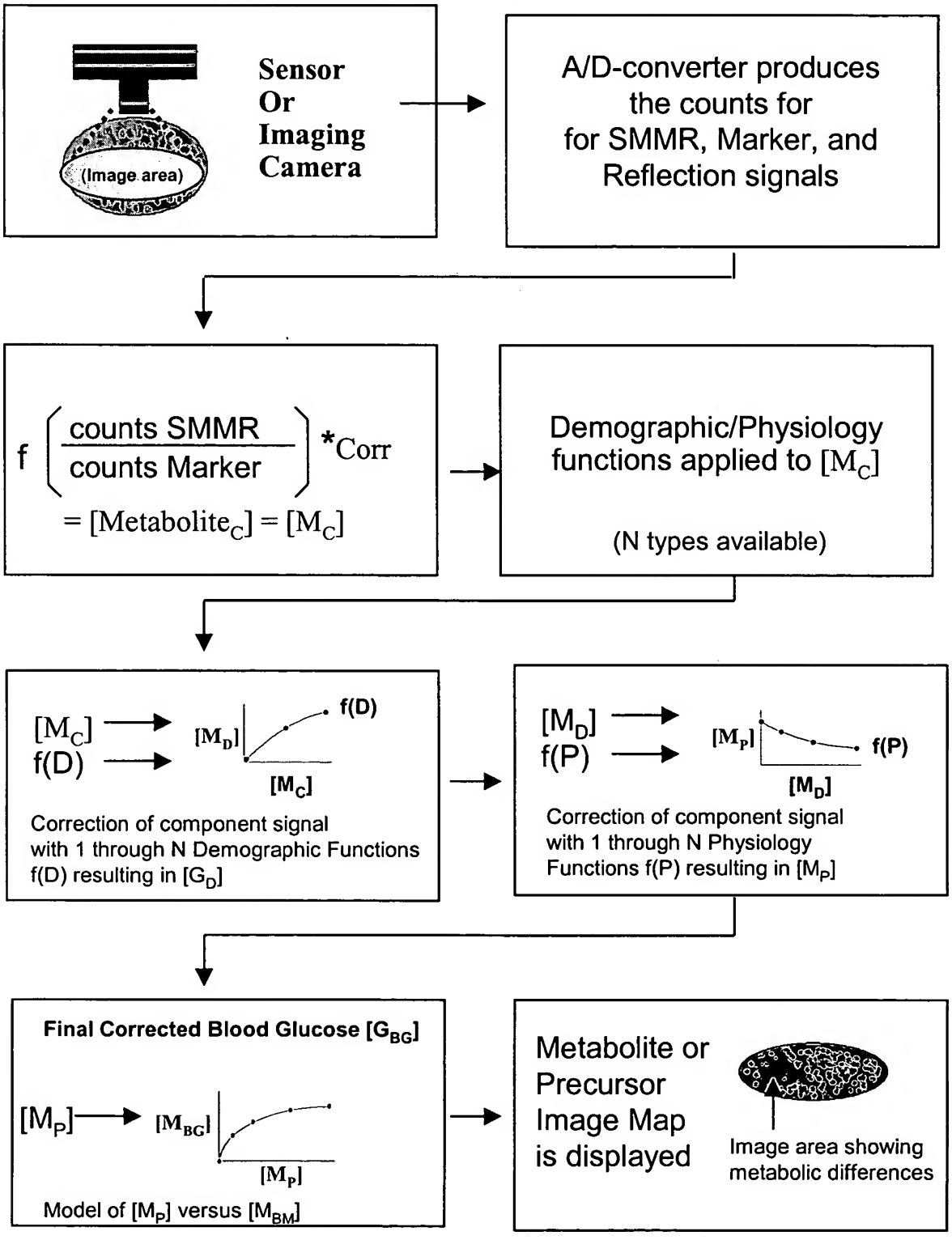

FIG. 37

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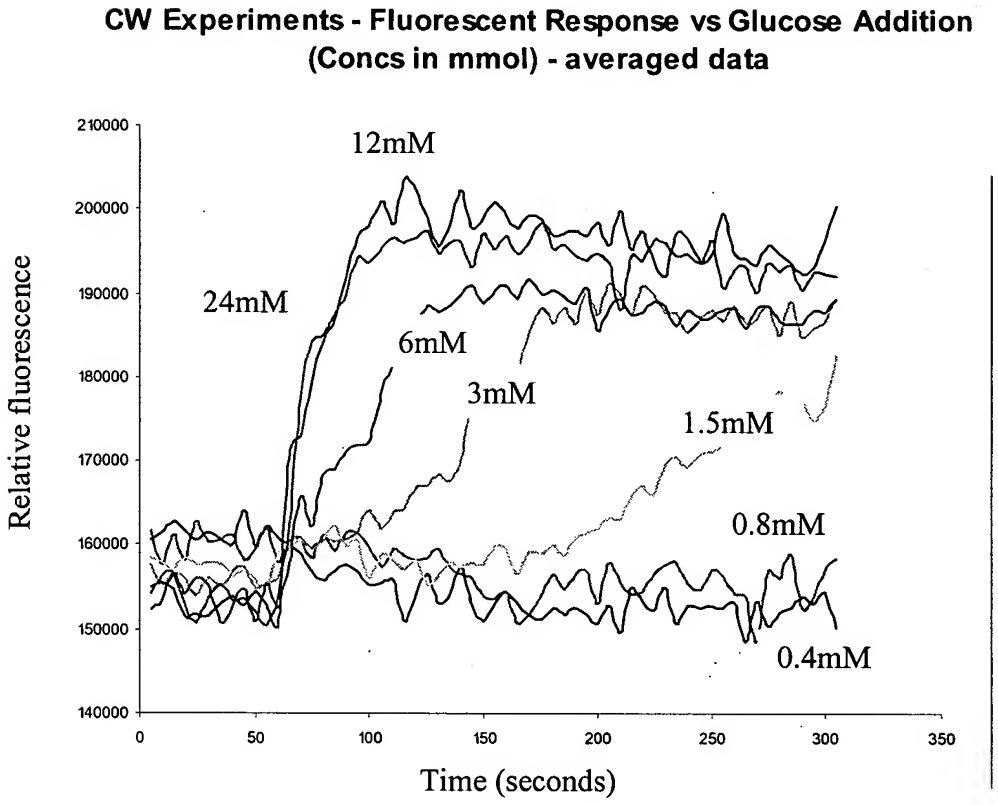
CW Experiments - Fluorescent Response vs Glucose Addition
(Concs in mmol) - averaged data
Relative fluorescence
210000
200000
190000
180000
170000
160000
150000
140000
0
50
100
150
200
250
300
350
Time (seconds)
12mM
24mM
6mM
3mM
1.5mM
0.8mM
0.4mM

FIG. 38

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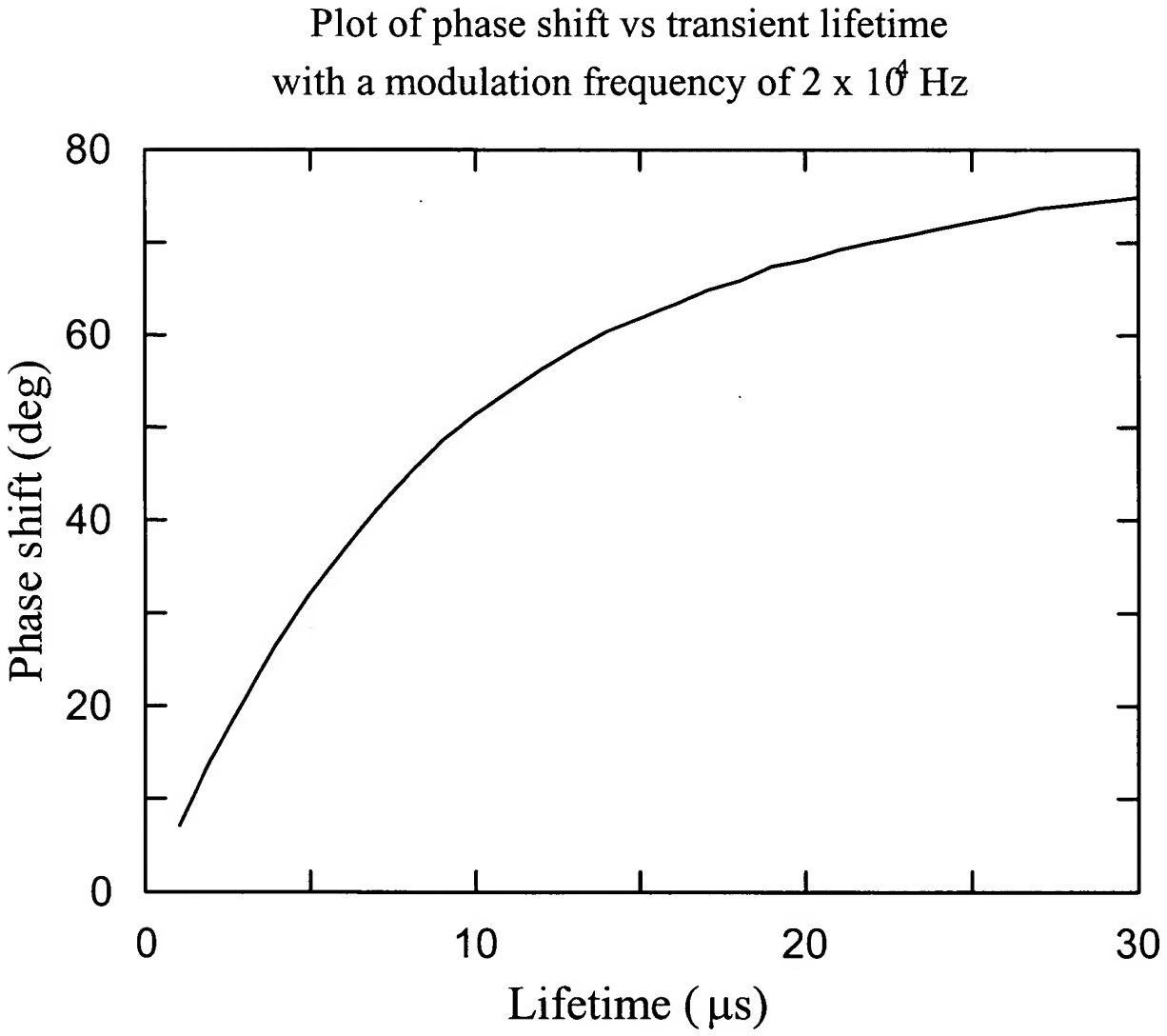
Plot of phase shift vs transient lifetime
with a modulation frequency of 2 x 10⁴ Hz
Phase shift (deg)
0
20
40
60
80
0
10
20
30
Lifetime (μs)

FIG. 39